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PRIMARY SYSTEMIC AMYLOIDOSIS

REPORT OF TWO CASES IN NEGROES, WITH SPECIAL REFERENCE TO CERTAIN HISTOLOGIC CRITERIA FOR DIAGNOSIS

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NEW ORLEANS

Primary systemic amyloidosis is a disease of unknown cause which involves tissues of mesenchymal origin, in contradistinction to secondary amyloidosis, which involves tissues of parenchymatous origin. It is a very uncommon condition. Koletsky and Stecher [1] in 1939 could collect from the literature only 26 cases, to which they added a personal case. The number now on record, including the additional case reported by Binford [2] and the 2 cases reported in this communication, is only 30.

The 2 cases of primary systemic amyloidosis herewith reported were encountered in 12,356 consecutive postmortem examinations at Charity Hospital of Louisiana at New Orleans from Jan. 1, 1930 to Jan. 1, 1940. Both patients were Negroes. During the same period 7,615 necropsies were performed on Negroes. Thus the incidence of the disease in general was 0.016 per cent, and the incidence in the Negro race was 0.026 per cent.

In addition to putting on record these 2 cases, which are apparently the first instances of primary systemic amyloidosis occurring in Negroes to be reported, our purpose in this communication is to point out certain histologic features of the disease which may prove helpful in diagnosis.

REPORT OF CASES

CASE 1.—A Negro woman 69 years of age was hospitalized, Nov. 5, 1937, for dyspnea, edema, lesions of the mouth and tongue and a purpuric rash. She had

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1. Koletsky, S., and Stecher, R. M.: Arch. Path. **27**:267, 1939.
2. Binford, C. H.: Arch. Path. **29**:314, 1940.

been in good health until 1934, when precordial pain first appeared. For the next ten months her blood pressure ranged from 220 systolic and 100 diastolic to 248 systolic and 120 diastolic. In September 1935 papules first appeared inside her mouth and on her tongue, and Sept. 22, 1936 she first observed a purpuric rash over the body; at this time the blood platelets numbered 118,000 per cubic millimeter. Dyspnea and edema appeared in April 1937, at which time the blood pressure was 170 systolic and 94 diastolic.

Fig. 1.—Heart treated with compound solution of iodine, showing diffuse amyloid infiltration of the pericardium, myocardium and endocardium (case 1).

The patient was well nourished but orthopneic. The important physical findings were petechial hemorrhages under both eyes and over the entire left forearm and anterior chest wall, edema over the abdomen and back, ascites and bilateral hydrothorax. An alternating pulse was present, and the electrocardiogram gave evidence of myocardial disease. The urine contained albumin (3 plus). Hematologic examinations gave negative results except for a leukocyte count of 14,600. The albumin content of the pleural fluid was less than 2.5 per cent.

The patient died of congestive heart failure Nov. 19, 1937. The main anatomic findings at necropsy were primary amyloidosis involving the heart, lungs, esophagus, stomach, small intestine and spleen. Incidental findings, on the basis of cardiac failure, were bilateral hydrothorax, chronic passive congestion of the liver and edema. In this and in the following case the description of the necropsy observations is chiefly limited to organs affected by amyloid infiltration.

The heart weighed 565 Gm. The right and left ventricular walls were dull grayish red and had a homogeneous translucent waxy appearance. The auricular walls, which measured 0.5 cm. in thickness, were rigid and did not collapse when opened. Both ventricular walls were markedly hypertrophied; the left wall measured 2.5 cm. in thickness and the right 1.0 cm. After the entire heart had been treated with compound solution of iodine (Lugol's solution), the ventricular and auricular musculature, endocardium, pericardium and papillary muscles showed marked diffuse infiltration with amyloid (fig. 1).

The lungs were rubber-like in consistency, and the lower lobes of both lungs showed evidence of compression atelectasis. The left lung weighed 320 Gm. and the right 300 Gm.

The wall of the esophagus, which was 0.5 cm. in thickness, was pale and translucent. The muscular wall of the stomach became gradually thicker as the pylorus was reached. Compound solution of iodine showed massive infiltration of the muscularis of both organs. The walls of the jejunum and ileum, which were diffusely thickened, measuring on the average 0.5 cm., were pale and translucent. The test with compound solution of iodine was uniformly positive for amyloid in the muscularis and patchily positive for this deposit in the mucosa.

Microscopic Examination.—A large amount of amyloid infiltration was present between the muscle fibers of the myocardium. The walls of the smaller vessels were markedly thickened by amyloid infiltration, which was continuous with that between the muscle fibers. Homogeneous areas were irregularly distributed, and the intensity of the staining reactions with hematoxylin-eosin and amyloid stains was also irregular.

Amyloid infiltration caused marked narrowing of the lumens of the alveolar capillaries and transformed the septums into irregular masses of amyloid. Balls and amyloid masses of bizarre shape emerged from the alveolar vessels into the alveoli. The larger vessels showed amyloid involvement, and some amyloid was also present around the bronchi.

The submucosa of the esophagus, stomach, jejunum and ileum appeared as an irregular band of amyloid. The mucosa of the ileum and jejunum showed a slight degree of amyloid infiltration. The serosa of the vessels and the interstitial tissue were involved in the amyloid process. The serosal fat was converted into bizarre-shaped amyloid rings.

The normal architecture of the spleen was diffusely replaced by amyloid substance. No amyloid could be demonstrated in the liver, which showed only mild passive congestion, or the kidneys, which showed uniform hyperplastic sclerosis of the afferent arterioles. The vessels of the thyroid gland showed amyloid infiltration. No changes were observed in the remaining organs.

Case 2.—A Negro man 60 years of age was hospitalized, Nov. 11, 1939, for dyspnea, hoarseness, which for ten months had been associated with a progressive cough and blood-tinged sputum, and edema of the ankles, which had been present for two months.

The patient was emaciated and dyspneic and could speak only in a whisper. Several firm irregular nontender subcutaneous nodules on the forearm and back

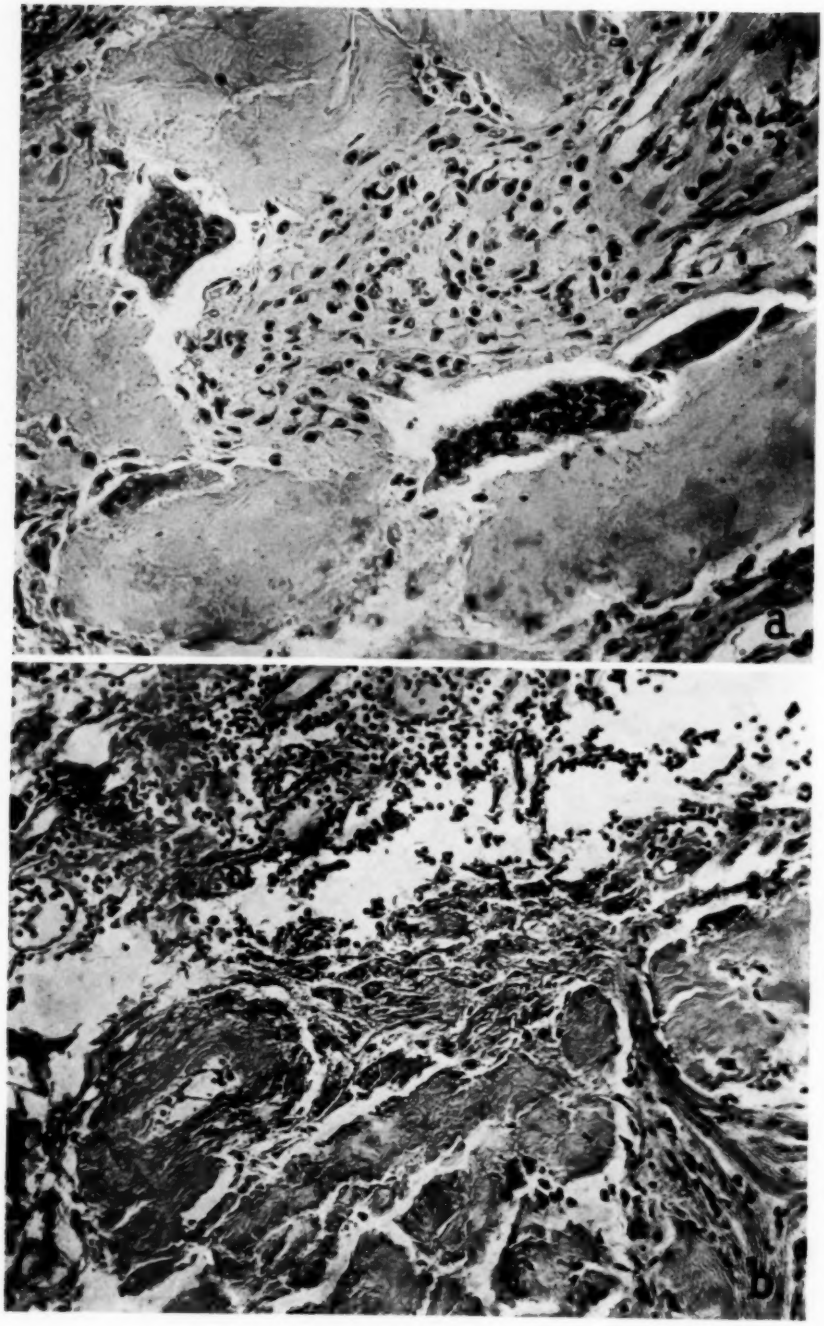

Figure 2

(See legend on opposite page)

moved on muscular contractions. A mass was present in the posterior pharyngeal wall, the vocal cords were paralyzed, and the arytenoid cartilages were swollen. The thyroid cartilage was prominent and fixed. The left posterior cervical chain of lymph nodes was enlarged. The blood pressure was 148 systolic and 88 diastolic. A systolic murmur was heard at the apex. Routine laboratory examinations revealed nothing significant. A biopsy of nodules on the arm was reported as showing amyloid infiltration.

Tracheotomy became necessary December 6, and the patient died as the anesthetic was being administered. The chief anatomic findings at necropsy were primary amyloidosis affecting the pharynx, larynx, trachea, esophagus, stomach, diaphragm, subcutaneous tissue, peritracheal and peribronchial tissues, great vessels, auricles of the heart and capsules of the adrenal glands.

The posterior pharyngeal wall, which was 2 cm. in thickness, was massively infiltrated with a homogeneous waxy material. When the larynx was opened anteriorly, three distinct masses were seen. One arose from the right ventricular fold and extended upward for 3 cm., involving the base of the epiglottis and projecting into the laryngeal cavity. Another, almost identical in size, appearance and position, arose from the left ventricular fold. The third and largest mass was situated in the midline of the posterior laryngeal wall; it was triangular, the apex being at the arytenoid cartilages and the base at the level of the fifth tracheal ring. The masses, which were homogeneous, gray and waxy, almost met in the midline and thus caused nearly complete laryngeal obstruction.

An irregular mass on the anterior external surface of the trachea extended from the fifth to the eleventh tracheal cartilage and fused with several tracheal lymph nodes. It merged laterally into an irregular infiltration surrounding the main bronchus and fused with the peribronchial lymph nodes. This mass in a downward extension uniformly involved the adventitial coats of the pulmonary vessels and the aorta, particularly the transverse arch, the wall of which measured 1 cm. in thickness.

The heart weighed 540 Gm. The right and left auricles, each of which measured 0.6 cm. in thickness, were rigid and thickened and were infiltrated with a homogeneous, yellowish gray waxy substance. The valves were unaffected except for a slight, apparently fibrotic thickening of the mitral and tricuspid valves. The ventricles were grossly unaffected.

Infiltration of the gastrointestinal tract was limited to the esophagus and stomach. An irregular, rather well demarcated infiltration, measuring 2.5 by 1.7 cm., was situated 10 cm. from the cardioesophageal junction, and a second, similar infiltration, measuring 3 by 2 cm., was situated 16 cm. from it. A similar nodule on the greater curvature of the stomach, 5 cm. from the pylorus, measured 3 by 3 by 2 cm.

The diaphragm presented several diffuse nodular areas in the region of the middle arcuate ligament. The capsules of the adrenal glands and the visceral

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EXPLANATION OF FIGURE 2

(*a*) Microscopic section of laryngeal nodules revealing irregular masses of amyloid and large irregular giant cells with small dense nuclei, which are distributed evenly throughout the cell (case 2).

(*b*) Microscopic section of the pulmonary artery, showing numerous lymphocytes, diffuse amyloid involvement of a small vessel and marked amyloid infiltration of adventitia (case 2).

pleura were positive for amyloid by the test of compound solution of iodine, but the remaining organs were all normal.

Microscopic Examination.—Although the epithelium of the posterior pharyngeal wall was normal, the underlying tissue was diffusely infiltrated with masses of amyloid of varying staining intensities. Lymphocytic foci were prominent. The walls of all the vessels were replaced by amyloid, in some instances to the point of obliteration of the lumens. In the larynx and trachea, in which similar findings were observed, a striking feature was the presence of a moderate number of large irregular giant cells with granular pink cytoplasm and regular dark-staining nuclei situated diffusely throughout the cell, without definite pattern (fig. 2*a*). A small number of plasma cells was also noted. The peritracheal and peribronchial tissue showed chiefly amyloid infiltration, which was located around but did not involve the cartilages.

The amyloid infiltration in the esophagus was located directly beneath the epithelium. A small amount was found in the vessels. In the areas in which amyloid, whether abundant or sparse, was present, marked lymphocytic infiltration and many plasma cells were observed. In the stomach the amyloid was confined to the muscularis, in which giant cells, large and small aggregations of lymphocytes, and plasma cells were present, as in the larynx and trachea. The muscle fibers of the diaphragm were atrophied by massive amyloid infiltration, and lymphocytic foci were present. The smaller vessels were markedly affected by amyloid infiltration.

The greatest amount of amyloid present in the heart was observed in the right auricle, the atrophied muscle bundles of which were separated by irregular masses of it. These masses stained with varying intensities. In some areas, in which the amyloid was less sparse, irregular foci of lymphocytes could be seen enmeshed in strands of amyloid. Plasma cells were also present. The smaller vessels were converted into homogeneous amyloid masses from which irregular streamers of amyloid were continuous with the interstitial amyloid substance. Although gross inspection and tests with compound solution of iodine had shown no evidence of amyloid in the ventricles, microscopic examination showed that these contained small amounts, which were confined to the small vessels or were slightly diffused between the muscle fibers. No atrophy of the muscle fibers could be seen. In the mitral valves were areas in which the collagen fibers were increased and other areas in which there were irregular globules of amyloid substance. In some areas a reticulated meshwork of hyalin-like strands contained lymphocytes. The tricuspid valve presented a similar but less marked picture.

Amyloid infiltration to a uniform degree practically replaced the adventitia of the ascending, transverse and descending arch of the aorta, was marked in the media but did not involve the intima. It seemed to emanate diffusely from the smaller vessels of the adventitia to form irregular globules. Many of these vessels were so diffusely involved that their lumens were actually occluded. The septums of the loose areolar tissue were transformed into "rigid" rings of amyloid. Foci of lymphocytes and a few irregular foreign body giant cells were present in the adventitia. The nerves were not involved. The right pulmonary artery, the aorta and the left pulmonary artery showed similar pictures except that the involvement of the media was minimal (fig. 2*b*).

The left pulmonary vein showed marked amyloid changes in the walls of the adventitial vessels, and the adjacent lymph nodes, in their sinuses. Distinct infiltrations of amyloid, with disappearance of the normal architecture of the lymph nodes, were observed wherever the adventitia of the vein encroached on the lymph nodes. The nerves were free of amyloid.

The interstitial tissue of the lung was thickened and showed occasional small globules of amyloid. Small amounts of amyloid in the vessels were confined to the adventitia.

The adrenal glands were normal except for the capsules, which showed irregular masses of amyloid apparently emanating from the smaller vessels. The vessels themselves were converted into amyloid. All the areolar and adipose supporting

Fig. 3.—Adipose tissue from the capsule of the adrenal gland, showing conversion of fat cells into "amyloid rings" (case 2).

tissue was transformed into amyloid and had the appearance of thickened rings (fig. 3).

The liver showed a small amount of chronic passive congestion, but the spleen, kidneys and other organs revealed no evidence of amyloid infiltration.

Reexamination of the biopsy specimens taken from the subcutaneous nodules on the arm revealed irregular masses of pink-staining homogeneous material which partially replaced the normal structure, many foci of lymphocytes, a few giant cells and plasma cells, and amyloid rings.

CLASSIFICATION OF AMYLOID DISEASE

On the basis of Lubarsch's [3] demonstration of a form which involved the mesenchymal tissues rather than the visceral organs, a more satisfactory classification of amyloid disease has been evolved. Many of the cases in the older reports [4] can now be placed in the group of primary amyloidosis.

Lubarsch also observed that the disease was distinguished both by its unusual anatomic distribution and its atypical staining reaction. The atypical staining reaction has been emphasized by other writers.[5] Weber and his associates,[5a] for instance, had negative results with iodine and congo red, as well as with methyl violet unless this was used in combination with oxalic acid. Other authors have observed varying intensities in the staining reaction and have pointed out that some portions of the amyloid substance stain well and others faintly or not at all. This observation has been substantiated in our own experience with methyl violet stains. It was also our experience that gross tissue has a marked affinity for iodine stain.

Magnus-Levy [6] pointed out that amyloid disease is rather frequently associated with multiple myeloma. Von Bornsdorf,[7] among others, has reviewed the literature on the localized form of the disease.

The classification of amyloid disease now generally accepted was advanced by Koletsky and Stecher [1] and by Reiman, Koucky and Ecklund.[5b] The cases are divided into four groups: (1) secondary amyloidosis; (2) primary amyloidosis; (3) amyloidosis with multiple myeloma; (4) localized amyloid tumors. The cases reported in this paper seem to fall into the second group.

COMMENT

In the first case which we have reported it is uncertain just how much the amyloid infiltration of the heart contributed to the cardiac failure which was the cause of death. Hypertension was first observed in the patient, who was 69 years old, six years before her fatal illness. At autopsy the heart weighed 565 Gm. and the kidneys showed hyper-

3. Lubarsch, O.: Virchows Arch. f. path. Anat. **271**:867, 1929.

4. (*a*) Wild, C.: Beitr. z. path. Anat. u. z. allg. Path. **1**:177, 1886. (*b*) Steinhaus, F.: Ztschr. f. klin. Med. **45**:375, 1902. (*c*) Ritter, E.: Virchows Arch. f. path. Anat. **192**:536, 1908. (*d*) Beneke, R.: Centralbl. f. allg. Path. u. path. Anat. **33**:240, 1922. (*e*) Königstein, H.: Arch. f. Dermat. u. Syph. **148**:330, 1925.

5. (*a*) Weber, F. P.; Cade, S.; Stott, A. W., and Pulvertaft, R. J. V.: Quart. J. Med. **6**:181, 1937. (*b*) Reiman, H.; Koucky, R., and Ecklund, C. M.: Am. J. Path. **11**:977, 1935. (*c*) Perla, D., and Gross, H.: ibid. **11**:93, 1935.

6. Magnus-Levy, A.: Ztschr. f. klin. Med. **126**:62, 1933.

7. von Bornsdorf: Arb. a. d. path. Inst. d. Univ. Helsingfors **7**:369, 1933.

plastic sclerosis of the smaller arterioles. Our own opinion, like Budd's [8] in his report of a similar case, is that the main cause of cardiac failure was essential hypertension. On the other hand, the amyloid infiltration of the cardiac muscle and the massive obliterating vascular infiltration of the lungs undoubtedly played a considerable role in the production of right and left ventricular failure.

The purpuric rash present at the time of death in our first patient had developed four years before and had apparently recurred at frequent intervals. In Lubarsch's second case, which resembled our own in other respects, the patient presented herself with petechial hemorrhages of the skin, and the condition was diagnosed clinically as thrombopenic purpura. The patient, a 66 year old woman, died of cardiac failure, and autopsy revealed amyloid involvement of the heart, tongue, gastrointestinal tract, esophagus, lungs and skin.

Our first patient also had red papules on the tongue, which in the light of the subsequent clinical course is suggestive of amyloid infiltration, although no biopsy was undertaken. Lingual involvement seems to be fairly common in typical amyloid disease and occasionally becomes extensive enough to be considered amyloid macroglossia. Pick [9] first pointed out its frequent occurrence, especially in association with sclerodermic manifestations, and its presence may be useful in making a clinical diagnosis of atypical amyloid disease. Such a diagnosis was made by Reiman and his associates [5b] and by Michelson and Lynch [10] and was confirmed by biopsy in both cases.

In our second case the dyspnea and gradual loss of voice of which the patient, a 60 year old Negro man, had complained for two years, were shown at necropsy to have been caused by obstruction of the laryngeal lumen by amyloid masses. The fact that the bulk of the amyloid deposits appeared in the form of multiple tumefactions of the larynx, pharynx and peribronchial tissues might suggest that this case falls into the class of localized amyloid tumors. On the other hand, the extensive mesodermal involvement, as manifested by the amyloid infiltration in the heart, esophagus, stomach, diaphragm, subcutaneous tissues and coats of the great vessels, puts the case in the class of primary amyloid disease, as does the atypical staining reaction.

In our first case the most striking microscopic changes were observed in the heart and lungs. The amyloid infiltration in the heart seemed to emanate from the smaller vessels, with secondary infiltration and atrophy of muscle fibers. This process is an almost constant finding in primary amyloidosis, although the degree of severity varies. The

8. Budd, J. W.: Am. J. Path. **10**:299, 1934.

9. Pick, L.: Klin. Wchnschr. **10**:1515, 1931.

10. Michelson, H. E., and Lynch, F. W.: Arch. Dermat. & Syph. **29**:805, 1934.

pulmonary findings were equally striking. Nearly all the alveolar vessels were involved, the vascular lumens were markedly narrowed and sometimes obliterated, and large globules of amyloid were numerous, and some were actually shed into the alveolar space. Similar findings were excellently illustrated by Reiman and his associates.[5b] In our own case the spleen was massively involved, which is not common in primary amyloidosis but which can occur.[11]

Involvement of the fibroadipose tissue was a prominent observation in both our cases and has been mentioned by others.[12] In our opinion, amyloid changes in adipose tissue are important. When they occur in the form of highly characteristic amyloid rings they are helpful in making a diagnosis from biopsy specimens.

The most constant microscopic changes in our second case were the numerous large irregular foreign body giant cells, the foci of lymphocytic infiltration and, in particular, the characteristic amyloid rings in adipose tissue. Because of the variability of the staining reactions of primary amyloidosis and difficulty of differentiating amyloid from ordinary hyalin, the finding of these three features, either alone or in combination, would seem to be of great value in making the diagnosis from a specimen secured for biopsy.

SUMMARY

Two cases of primary amyloid disease, the first of this disease in the Negro race to be put on record, are reported from Charity Hospital of Louisiana at New Orleans. The incidence of the disease over a ten year period was 0.016 per cent in all patients coming to autopsy and 0.026 per cent in all Negro patients coming to autopsy.

In the first case death was due to cardiac failure, with extensive amyloid involvement of the heart, lungs and gastrointestinal tract. In the second case death was due to laryngeal obstruction by amyloid tumor masses, with amyloid involvement of the pharynx, peribronchial and peritracheal tissues, heart, great vessels, esophagus, stomach, diaphragm, lungs and subcutaneous tissues.

Special stains for amyloid show variability in staining reaction and in intensity.

Significant histologic criteria for diagnosis are foreign body giant cells, foci of lymphocytic infiltration and amyloid rings, in addition to irregular masses of amyloid. These criteria are particularly useful when diagnosis must be made from a specimen secured for biopsy. They are also useful, either singly or in combination, because of the possibility of mistaking amyloid for hyalin and because of the variability of staining reactions with special stains.

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11. DeNavasquez, S., and Treble, H. A.: Brain **61**:116, 1938. Lubarsch.[3]

12. Strauss, A.: Virchows Arch. f. path. Anat. **291**:219, 1933. Lubarsch.[3] Königstein.[4e]

VISUALIZATION OF VITAMIN A IN RAT ORGANS BY FLUORESCENCE MICROSCOPY

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Much information on the physiology of vitamin A has been collected by determining the vitamin A contents of different organs of experimental animals. This has been done by chemical, spectroscopic and biologic methods.[1] To visualize vitamin A in organs for histologic examination would present definite advantages. The exact localization of the vitamin within the organ could be determined; further, the presence of minute quantities might be demonstrable, if vitamin A is localized within a certain structure of the organ.

The fading green fluorescence characteristic of vitamin A [2] in oil has been made the basis for histologic visualization.[3] Querner [3a] described green fluorescent inclusions fading on irradiation in the epithelial cells of the liver, adrenal and pituitary gland; he maintained that this fluorescence was due to the presence of vitamin A. A similar method has been used by other investigators.[4] Hirt and Wimmer [3b] demonstrated various vitamins in living animals by fluorescence microscopy and described the distribution of vitamin A in different

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1. (*a*) Moore, T.: Biochem. J. **25**:275, 1931. (*b*) Baumann, C. A.; Riising, B. M., and Steenbock, H.: J. Biol. Chem. **107**:705, 1934. (*c*) McCoord, A. B., and Luce-Clausen, E. M.: J. Nutrition **7**:557, 1934. (*d*) Edisbury, J. R.; Morton, R. H.; Simkins, G. W., and Lovern, J. A.: Biochem. J. **32**:118, 1938. (*e*) Chevallier, A., and Choron, Y.: Compt. rend. Soc. de biol. **120**:1223, 1935.

2. Peacock, P. R.: Lancet **2**:328, 1926. Morgan, R. S., and MacLennan, K.: Biochem. J. **22**:1514, 1928.

3. (*a*) von Querner, F.: Klin. Wchnschr. **14**:1213, 1935; Anat. Anz. **78**:289, 1934. (*b*) Hirt, A., and Wimmer, K.: Klin. Wchnschr. **18**:733 and 765, 1939; **19**:123, 1940. (*c*) Popper, H.: Proc. Soc. Exper. Biol. & Med. **43**:133, 1940.

4. (*a*) von Jancsó, N., and von Jancsó, H.: Biochem. Ztschr. **287**:289, 1936. (*b*) Schairer, E.; Rechenberger, J.; Gockel, H., and Patzelt, K.: Virchows Arch. f. path. Anat. **305**:360, 1939.

organs. The antimony trichloride reaction has also been used for the histologic demonstration of vitamin A.[5]

In this paper the histologic distribution of vitamin A in normal, vitamin A–deficient and hypervitaminotic rats is described. Additional evidence is offered for the specificity of the histologic technic. The conditions and the morphologic picture in depletion and replacement are described.

METHODS

Histologic Technic.—Fluorescence microscopy in general is described in the monographs of Haitinger[6] and Radley and Grant.[7] Briefly, light rich in ultraviolet rays is filtered through an ultraviolet filter (Corning glass color filter no. 584) and a 5 per cent copper sulfate solution. An ordinary microscope is used; above the eye piece is a brown filter to absorb the ultraviolet rays.

The tissue is fixed in solution of formaldehyde U. S. P. (1:10) for not more than six hours. Frozen sections mounted in water are examined shortly after cutting.

The substance hereinafter referred to as vitamin A induces a striking green fluorescence which fades while being observed in ultraviolet rays. Other green fluorescence which does not fade should not be confused with that of vitamin A. The fat-soluble vitamin A is, with few exceptions, bound to lipoids which are morphologically demonstrable by either the common fat stains or by phosphin 3R, a fluorescent dye. The green fluorescent tissue constituents which do not fade are not vitamin A.

A more detailed description of the method is given in another paper.[8]

Preparation of Animals.—Altogether 237 rats were examined. The normal animals (27 rats) were on a commercially available mixed diet,[8a] to which vitamin A was added. The avitaminotic rats received a diet free of vitamin A according to the U. S. P. XI standards, revised 1937. When not otherwise stated the animals were weaned and placed on the vitamin A–free diet at 21 days of age. Their weights ranged between 30 and 40 Gm. Their mothers received the stock diet.

In the restoration experiments, animals which did not show gross pathologic signs were used. The rats were still gaining some weight and looked well. There were no indications of disturbed intestinal absorption. Vitamin A was administered by mouth or by stomach tube or parenterally.

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5. Joyet-Lavergne, P.: Protoplasma **28**:131, 1937; Compt. rend. Acad. d. sc. **200**:346, 1935.

6. Haitinger, M.: Fluorescenz-Mikroskopie, Leipzig, Akademische Verlagsgesellschaft, 1938.

7. Radley, J. A., and Grant, J.: Fluorescence Analysis in Ultra Violet Light, ed. 3, New York, D. Van Nostrand Company, Inc., 1939.

8. Popper, H.: Arch. Path. **31**:766, 1941.

8a. The diet was stated to contain riboflavin concentrate, dried skim milk, brewers' dried yeast, wheat germ, barley malt, carotene, dried beet pulp, meat meal, soybean oil meal, molasses, corn grits, cereal feed (from corn and wheat), cod liver oil, 1 per cent steamed bone meal and 1 per cent iodized salt.

In the hyperavitaminosis A experiments,[9] 12 young adults, weighing between 175 and 225 Gm., received daily for thirty days intraorally 15,000 international (U. S. P. XI) units of vitamin A concentrate. The rats gained little or no weight during this period.

Chemical Analyses.—The analyses of the livers for vitamin A were made by the method of Guilbert and Hart.[10]

HISTOLOGIC DISTRIBUTION OF VITAMIN A IN THE RAT UNDER VARIOUS CONDITIONS

Liver.—The livers of rats on the diet rich in vitamin A (fig. 1 *B*) showed vitamin A fluorescence in varied distributions. In the cells the fluorescence was seen in small lipoid droplets on the edges of the cytoplasm. These lipoid droplets marked the boundary between the liver cells and the sinusoids. The cytoplasm itself had a green fading but dimmer fluorescence, indicating a diffuse distribution of minute amounts of vitamin A, which was not bound to morphologically demonstrable lipoids. After fading of the vitamin A fluorescence, the cytoplasm showed a dim blue relatively homogeneous fluorescence. The lipoid droplets on the edges of the cells were no longer visible. If larger fat droplets were present in the liver cells, they showed the vitamin A fluorescence in varying amounts. After fading, these larger fat droplets still stood out in the dim blue of the cytoplasm. They showed a bluish white fluorescence, much like fat droplets that had never contained vitamin A.

The Kupffer cells contained small lipoid droplets rich in vitamin A. These droplets were located close to the nuclei, thereby giving the Kupffer cells a bulky appearance in fluorescence light. This was unlike the winglike fluorescence of human Kupffer cells.[8] Irregularly shaped bodies of red nonfading fluorescence were seen in Kupffer and liver cells. Their number was increased in older animals. They may have represented lipofuscin[11] or some form of another vitamin.[3b]

Vitamin A Deficiency: In the livers of animals deficient in vitamin A the green fading fluorescence was absent (fig. 1 *A*). Only the dim blue of the cytoplasm was visible. Fat droplets showing the bluish white ultraviolet-stable fluorescence were occasionally present, especially in areas of fatty infiltration. Reddish inclusions were seen in Kupffer and liver cells. The picture resembled that of vitamin A–containing liver after irradiation of the tissue with ultraviolet rays, or after a short treatment with alcohol, or after prolonged fixation. The difference between the green fluorescence of normal liver and the dim blue of avitaminotic liver was seen even with the naked eye.

The livers of stock animals showed many variations in their vitamin A fluorescence. Livers of animals kept on a diet low in vitamin A showed a small

9. (*a*) Harris, L. J., and Moore, T.: Biochem. J. **22**:1473, 1928. (*b*) von Drigalski, W.: Klin. Wchnschr. **12**:308, 1933. (*c*) Moll, T.; Domagk, G., and Laqueur, F.: ibid. **12**:456, 1933. (*d*) Domagk, G., and von Dobeneck, P.: Virchows Arch. f. path. Anat. **290**:385, 1933.

10. Guilbert, H. R., and Hart, G. H.: J. Nutrition **8**:25, 1934.

11. Hamperl, H.: Virchows Arch. f. path. Anat. **292**:1, 1934.

amount of the fluorescence in a heterogeneous distribution. The vitamin A was located around the central veins, and the Kupffer cells retained relatively more than the liver cells. The distribution of fat paralleled that of vitamin A fluorescence, although many lipoid droplets were free of the vitamin. In livers with fatty infiltration the fat droplets showed moderate amounts of vitamin A fluorescence, dependent on the vitamin A content of the liver.

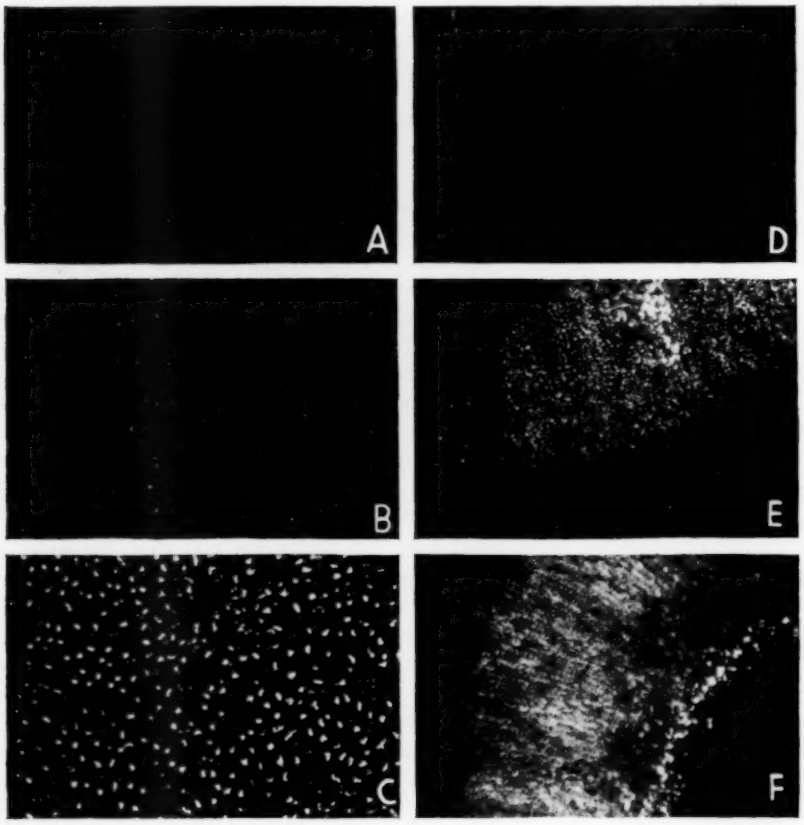

Fig. 1.—Fluorescence photomicrographs: *A*, liver of rat deficient in vitamin A; a central vein is seen in the center of the field. *B*, liver of a normal rat; moderate amounts of vitamin A fluorescence are present in the Kupffer cells and at places in fine droplets at the edges of the liver cells. *C*, liver of rat made hypervitaminotic with vitamin A; marked vitamin A fluorescence is seen in the Kupffer cells, which apparently are "proliferated"; there are moderate amounts of the fluorescence in small lipoid droplets within the liver cells. *D*, adrenal of a vitamin A–deficient rat. *E*, adrenal of well fed rat; moderate amounts of vitamin A fluorescence are seen in lipoid droplets of the fascicular layer. *F*, adrenal of a rat made hypervitaminotic with vitamin A; there is striking vitamin A fluorescence of the lipoid droplets of the fascicular layer; the lipoid droplets of the narrow glomerular layer (hardly recognizable shadow at the outer surface) are free of vitamin A fluorescence.

Hypervitaminosis: According to Davies and Moore,[12] after a rat has been fed excessive amounts of vitamin A, the liver may contain enough of the vitamin to maintain the rat for a century. The livers of our hypervitaminotic rats showed a striking green fluorescence—impressive even when seen with the naked eye. The Kupffer cells were loaded with vitamin A (Hirt and Wimmer [3b]); they were increased in size and apparently proliferated (fig. 1 *C*); the time of complete fading was a matter of several minutes. After fading, dim white vacuoles remained in the Kupffer cells, indicative of their high fat content,[13] which was seen in sections stained with phosphin 3R. The vitamin A fluorescence of the liver cells was not much higher than that in well fed normal rats; it was seen in small lipoid droplets along the edges of the cells.

Newborn State: The livers of newborn rats revealed only traces of vitamin A in Kupffer and liver cells. Young animals had a smaller storage than older ones.[1b]

Adrenal Gland.—The adrenal glands of the stock animals contained large amounts of vitamin A in small lipoid droplets in the epithelial cells of the fascicular layer of the cortex. This confirmed the observation of Querner.[3a] The droplets filled the cells almost completely; the nuclei did not contain them. The outer portion of the fascicular layer showed the highest amount of vitamin A (fig. 1 *E*). After fading, the lipoid droplets showed a faint blue fluorescence. Phosphin 3R stained these anisotropic lipoid droplets. The distribution of the lipoids in the fascicular layer was similar to that of the vitamin A fluorescence. The glomerular layer contained anisotropic lipoid droplets which stained with phosphin 3R. A boundary zone, marking the glomerular and fascicular layers, supposedly concerned with the regeneration of epithelial cells,[14] was free of lipoids. Only the fascicular layer revealed vitamin A fluorescence, although both the glomerular and the fascicular layer contained lipoids. This fact suggests a specific affinity of vitamin A for certain lipoids and against a nonspecific solubility in lipoids.

The inner zone of the cortex was normally free of lipoids and vitamin A. Young hooded rats and mice, animals in which an x zone had been described,[14] did not show a recognizable x zone as determined by a change in the vitamin A picture. The medulla did not contain vitamin A. It showed dull green nonfading fluorescent inclusions.

The adrenals of vitamin A–deficient rats did not show vitamin A fluorescence (fig. 1 *D*). The ultraviolet-stable blue fluorescence of the cytoplasm resembled that of the normal rat after fading of the vitamin A fluorescence. If the deficiency was not far advanced the distribution of fat was similar to that of the stock animal. In the more advanced deficiency lipoids were no longer present. In rats with low vitamin A reserves a homogeneous thin zone on the outer margin was the only part of the fascicular layer that contained vitamin A. The patchy distribution often seen in the human adrenal was not encountered in the rat's gland.

In the adrenals of hypervitaminotic rats the fluorescence was much more extensive; it extended almost to the medulla (fig. 1 *F*). The glomerular layer and the medulla were free of vitamin A.

Kidney.—The kidneys of the control rats contained small amounts of vitamin A in cortex and medulla. In the interstitium between the cortical tubules there were fine granules showing vitamin A fluorescence, apparently localized in the

12. Davies, A. W., and Moore, T.: Biochem. J. **28**:288, 1934; **29**:147, 1935.

13. Fasold, H.: Ztschr. f. d. ges. exper. Med. **94**:35, 1934.

14. Grollman, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

endothelial cells of the capillaries. The fluorescence of these granules faded rapidly and could easily be overlooked; it was seen better in thick sections. These granules were not visualized by phosphin 3R, and they were not doubly refractile. The granules appeared in a nephron-bound distribution. Capillaries belonging to single nephrons showed the granules, while those of the surrounding nephrons did not.

In the capillary aggregations localized at the corticomedullary boundary, vitamin A was found in the capillary endothelial cells. Some of these aggregations were free of vitamin A; others contained much. In the medulla, vitamin A was found in the endothelium of the capillaries, but greater quantities were seen in lipoid droplets (demonstrable by phoshin 3R) in the stroma of the papilla.

The kidneys of the avitaminotic animals contained no vitamin A. In hypovitaminosis, the medulla was free of vitamin A, although the lipoid droplets were still demonstrable by phosphin 3R and the vitamin A fluorescence of the cortical interstitium was visible.

In hypervitaminosis the vitamin A content of the kidney was increased above normal in all the mentioned locations (fig. *2 A*). The nephron-bound storage was less marked, and, in addition, the proximal convoluted tubules showed vitamin A fluorescence. The fluorescence of the tubules was probably due to renal excretion.[15]

The vitamin A distribution in the kidney varies markedly in different species. The dog's kidney contains vitamin A in medium-sized lipoid droplets in the convoluted tubules. The normal human kidney shows no vitamin A fluorescence.

Lung.—The normal lung contained small quantities of vitamin A in the alveolar septums, apparently in the capillary epithelium and the interstitial cells (fig. *2 B*). Accumulations were seen in the perivascular tissue and in the subserosa of the pleura. Phosphin 3R stained the site of the vitamin A fluorescence in the subserosa. In the epicardium vitamin A was visible in a similar distribution.

In hypovitaminosis and in avitaminosis the lung was free of vitamin A. In hypervitaminosis very much vitamin A fluorescence was seen and even the epithelial cells of the alveoli contained vitamin A–carrying lipoids (fig. *2 C*).

Ovary.—The normal rat ovary showed vitamin A in two areas. The interstitial cell cords contained rather high amounts in large anisotropic lipoid droplets, which were stained by phosphin 3R. It was also seen in the corpora lutea, where it appeared in fine droplets, which did not show so strikingly double refraction (fig. *2 D*). After fading of the vitamin A fluorescence, a brown fluorescent fine granular pigment was seen, which increased in amount with age. The graafian follicles were free of vitamin A. In avitaminosis the ovary was free of vitamin A, whereas the brown granular pigment was still present.

Fat Tissue.—The fat tissue and the fat cells of different organs showed small varying amounts of the green fading fluorescence.[1d] After fading of this fluorescence, a darker green stable fluorescence remained. This should not be confused with that of vitamin A, as it is also present in vitamin A–deficient animals. The smaller fat droplets showed more vitamin A fluorescence than the surrounding larger droplets. There were great variations in the several fat depots of the body. No obvious relation with the vitamin A content of the liver or that of the adrenal was apparent.

Eye.—In the eye of the albino rat vitamin A fluorescence was seen in the rod and cone layer of the retina and in the pigment layer in the form of strongly

15. Boller, R., and Brunner, O.: Klin. Wchnschr. **15**:1106, 1936.

fluorescent fine droplets (Jancsó and Jancsó [4]), which were not doubly refractile. Vitamin A was always found in the light-adapted eye. It did not completely disappear during vitamin A deficiency, no matter how depleted the animal was, and was even seen in small amounts in ulcerated eyes. Visual purple was seen in rat

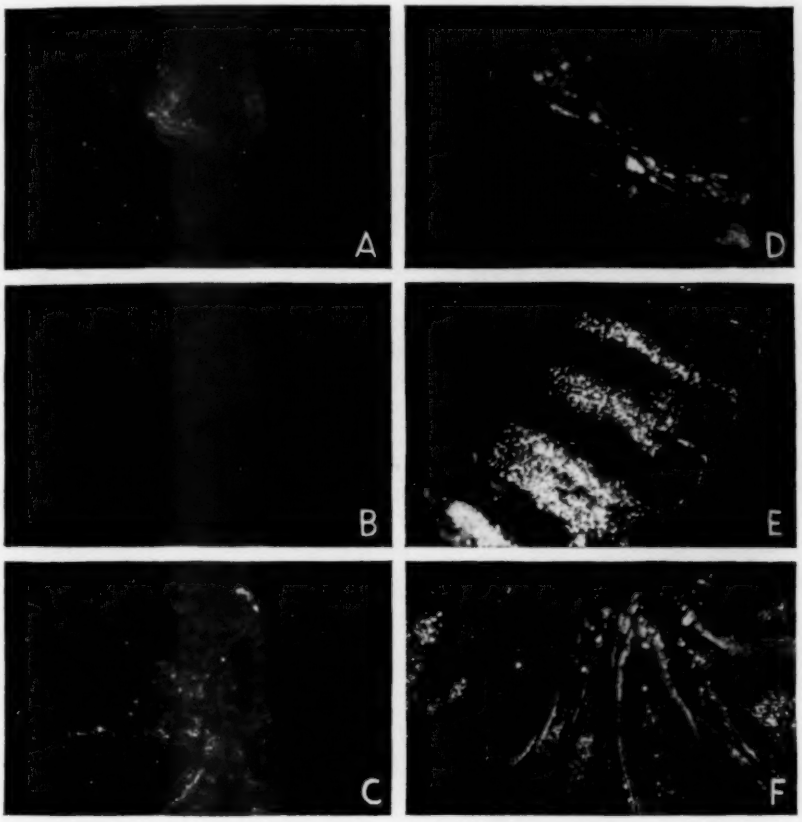

Fig. 2.—Fluorescence photomicrographs of rats' organs: *A*, kidney in hypervitaminosis A; fine droplets revealing vitamin A fluorescence are seen in the intertubular interstitium (capillary endothelium); there is vitamin A fluorescence of the tubular epithelium. *B*, normal lung; a few droplets showing vitamin A fluorescence are present in the alveolar septums, with a moderate accumulation in the perivascular tissue. *C*, lung in hypervitaminosis; many droplets carrying vitamin A are seen in the alveolar septums and in the perivascular tissue; some are also seen in the alveolar epithelium. *D*, ovary; the interstitial cell cords are rich in vitamin A fluorescence; the corpora lutea show slight amounts. *E*, jejunum after an oral intake of 8,000 international units of vitamin A; the epithelial lining of the villi is rich in vitamin A fluorescence and the lamina propria reveals high amounts; there is an accumulation of vitamin A fluorescence in the lacteals, especially in the submucous layer. *F*, jejunum after an oral intake of 5,000 international units of vitamin A; strong vitamin A fluorescence is shown by droplets within the lumen and moderate fluorescence by the epithelial lining and by cells in the center of the villi.

eyes in all stages of vitamin A deficiency,[16] although some[17] believe that in severe deficiency the eye loses its ability to form the substance. The investigation of the eye for vitamin A fluorescence during different states of light adaptation is still in progress.

Intestine.—The gastrointestinal tract of the normal rat did not reveal vitamin A fluorescence except in fat cells in various layers and in the subperitoneal layer, which contained vitamin A in a distribution similar to that of other serous membranes.

When vitamin A was fed in sufficient amounts, its fluorescence was seen in the lumen of the stomach and intestine. In the duodenum and upper part of the jejunum it was found in the wall; probably it is absorbed there. The greater part of the absorption took place in the tall thin villi. During absorption, vitamin A fluorescence was seen within the epithelial cells of the villi, in thin streaks at the adjacent edges of the epithelial cells, passing from the lumen to the lamina propria. In the lamina propria of the villi the vitamin was visible in one of two forms, (*a*) in granules seemingly carried by cells or (*b*) in threadlike streams in the lacteals, especially at the base of the villi. In larger lacteals it was traced from the submucosa through to the subserous layer (fig. 2*E* and *F*). After fading, the fat appeared as dim blue-white droplets. In the stroma of the villi there were at times whitish fluorescent nonfading granules. Such granules should not be confused with vitamin A. Phosphin 3R showed the lipoids in a distribution similar to that of vitamin A.

The histologic picture suggested lymphatic absorption of vitamin A.

Miscellaneous Organs.—The pituitary showed vitamin A fluorescence in the pars intermedia in some cases. The brain tissue itself was free of vitamin A. The meninges, however, showed it in a distribution similar to that seen in serous membranes.

The spleen did not show vitamin A fluorescence, although lipoid droplets were demonstrable.

The testicles, thyroid gland, thymus, lymph nodes, oviduct, tongue, skin and muscle tissue did not reveal vitamin A fluorescence.

EVALUATION OF FLUORESCENCE MICROSCOPY AS A HISTOLOGIC METHOD FOR VISUALIZATION OF VITAMIN A

The salient results of the histologic examinations were: high amounts of the green fading fluorescence in rats with hypervitaminosis A; moderate amounts in stock animals; none in deficient rats outside the retina and pigment coat of the eye. In order to support the thesis that the green fading fluorescence was due to the presence of vitamin A and the lack of the fluorescence was due to the absence of vitamin A, feeding experiments were performed. To prove the specificity of the fluorescence for vitamin A, it had to be determined what substance would recreate the green fading fluorescence in tissues which were made free of this fluorescence.

Experiments with Various Substances to Determine the Specificity of the Fluorescence.—In the feeding experiments, growing rats in early

16. Holm, E.: Am. J. Physiol. **73**:79, 1925.
17. Tansley, K.: Proc. Roy. Soc., London, s.B **114**:79, 1933.

vitamin A depletion were used. The organs, save the eye, were histologically free of vitamin A fluorescence, as determined in 21 animals.

(*a*) Vitamin A: After vitamin A (more than 200 international [U. S. P. XI] units) in corn oil had been fed the rats, vitamin A fluorescence reappeared in the liver and other organs. A detailed description of replacement experiments is included later in this paper. Generally, the amount of the restored green fluorescence depended on the amount of vitamin A fed. The amount of the green fading fluorescence in the liver was roughly in agreement with the amount of vitamin A chemically determined in the same organ. Six different vitamin A concentrates varying in strength from 10,000 to 1,000,000 international (U. S. P. XI) units per gram and diluted (10,000 or 1,000 international units per gram) in solvents such as percomorph liver oil or vegetable oil were examined for their fluorescence both in thin aqueous emulsions and in the organs of depleted rats after feeding. One of the concentrates examined had all of the vitamin A in the esterified form, and another, in the uncombined form; both showed the same green fading fluorescence.

(*b*) Carotene: Carotene and beta carotene crystals were dissolved in oil in water emulsions and examined for fluorescence. Neither showed the striking green, quickly fading fluorescence of vitamin A. Instead, in solutions containing more carotene than an equivalent of 700 international units of vitamin A per gram, they showed a dim green, rather slowly fading fluorescence. Vitamin A gave the green fading fluorescence in oil solutions containing more than 100 international units of vitamin A. The reduced fluorescence of carotene is illustrated by the fact that, unlike the fluorescence of vitamin A, it is concealed after methylene blue staining. As discussed later in detail, the feeding of carotene to vitamin A–deficient rats produced the typical green fading fluorescence of vitamin A. Its distribution in the organs was the same as that after feeding vitamin A.

Up to the present time, no other substance that we have fed has produced the characteristic fluorescence in the organs of the vitamin A–deficient rat.

(*c*) Oils: Vitamin A was localized in the tissue lipoids. Therefore the lipoids in the diet were examined. The following oils were fed (1 cc. twelve hours before the examination): corn oil, olive oil, linseed oil, peanut oil, cottonseed oil and lard. None of the samples contained vitamin A as determined by their fluorescence or by the Carr-Price reaction. None of them produced the green fluorescence in rat organs after being fed to 6 depleted rats.

(*d*) Other Vitamins: Although the vitamin A–free diet used contained the other vitamins in sufficient quantity, a borderline deficiency

or a combination deficiency of another vitamin might be considered as a contributing cause for the loss of the green fading fluorescence. Riboflavin [18] was fed to 2 vitamin A–deficient rats, 5 and 8 mg., respectively —to one twelve hours before killing, to the other in two portions, nineteen and six hours before killing. The following other vitamins were fed each to an avitaminotic rat in two doses, nineteen and six hours before killing; 12.6 mg. of thiamine hydrochloride, 13 mg. of ascorbic acid, 7 mg. of nicotinic acid and 5,000 international (U. S. P. XI) units of viosterol. In no case was green fading fluorescence found in the organs of these rats. After feeding a vitamin K preparation, the typical green fading fluorescence appeared, but the preparation was checked and found to contain some vitamin A.

The examination of vitamin B_2, or riboflavin, was important, as riboflavin has a well known green ultraviolet-labile fluorescence. When riboflavin was dissolved in the aqueous phase of a water in oil emulsion, droplets were seen to impart a green fading fluorescence under the microscope similar to that of vitamin A. Unlike vitamin A fluorescence, vitamin B_2 fluorescence was quickly destroyed by reducing agents. The green fading fluorescence in tissues was not affected by reducing agents. Hirt and Wimmer [3b] assumed that a part of the green fading fluorescence of tissues observed in vital microscopic examinations was due to the presence of vitamin B_2. Since the green fading fluorescence in fixed tissues is dependent on the alimentary intake of vitamin A and since it is independent of the intake of vitamin B_2, it is concluded that the fluorescence studied by us is not due to the presence of vitamin B_2.

Regarding the riboflavin fluorescence, several other points should be made. It is possible that riboflavin is present in tissues in a protein-bound form which is not influenced by reducing agents and which may or may not show fluorescence. Our results do not eliminate the possibility that a part of the green fluorescence seen in vital microscopic examinations, where other filters are or were possibly used, may be due to vitamin B_2. Also, the water-soluble vitamin B_2 may have been lost from the tissues during the fixation and preparation of the frozen sections.

We also found that rats deficient in vitamins other than vitamin A, such as B_1, B_2 [19] and D, and guinea pigs deficient in vitamin C possessed the usual green fading fluorescence in their organs.

Comparison Between the Histologic and the Chemical Method.—Out of the animals discussed in the following section, 71 rat livers were selected for chemical analysis for vitamin A in order to compare the

18. The various vitamin preparations were furnished by the Abbott Laboratories, North Chicago, Ill.; Mead Johnson & Co., Evansville, Ind., and Dr. A. L. Pirk of Hoffmann-La Roche Inc., Nutley, N. J.

19. Dann, W. J., and Moore, T.: Biochem. J. **25**:914, 1931.

results of the histologic and chemical methods. The livers were examined because of the uniformity of their distribution of vitamin A. Chiefly those of animals on the borderline of deficiency, in which only a small amount of vitamin A fluorescence was seen, were selected, to show the limit of the histologic method.

Vitamin A fluorescence was always seen in the liver if more than 10 international units of vitamin A was present in the organ (about 2 units per gram). At times the fluorescence was present in a liver containing but 0.5 unit per gram. In 8 cases complete agreement between the chemical and the histologic results was not found. In 4 of these the chemical test failed to show vitamin A, though traces of the fluorescence were seen. Far advanced depletion was noted, and the remaining vitamin A stores were scattered, with only a few vitamin A–containing cells recognizable in a field. This heterogeneous distribution may explain the negative chemical tests. In the 4 other cases the Carr-Price test gave a faint blue reaction, but there was no vitamin A fluorescence. In 2 of these cases the rats had been fed vitamin A only four hours previously and possibly the blood in the liver contained vitamin A. High blood levels of vitamin A have been reported four hours after feeding vitamin A.[20]

PHYSIOLOGIC EXPERIMENTS

Depletion in Growing Rats.—The rate of depletion varied with the age of the animal.[21] Older animals had stored more vitamin A, and their depletion required more time.

The time of depletion in growing animals (38 rats) depended on the time that elapsed between the day of weaning and the day the rat was put on the vitamin A–free diet. During this time the vitamin A storage was markedly increased, postponing the moment of complete depletion. When the animals were given a vitamin A–free diet immediately on weaning (twenty-first day), the rats were depleted at approximately the fifteenth day. Animals put on a vitamin A–free diet three days after weaning retained their stores as late as the twenty-eighth day.[22]

In all further discussions the animal is considered depleted if all the organs were free of vitamin A save the retina and pigment coat of the eye (which always showed vitamin A fluorescence if the eye was light adapted).

In the liver the depletion started around the periportal veins and progressed gradually until the tissue around the central vein was free.

20. Clausen, S. W.: J. A. M. A. **101**:1348, 1933.
21. Dann, W. J.: Biochem. J. **28**:2141, 1934.
22. Davies, A. W., and Moore, T.: Biochem. J. **31**:172, 1937.

The liver cells lost their vitamin A before the Kupffer cells. In the liver cells, the fine droplets adjacent to the Kupffer cells were the last to lose their fluorescence. Therefore, the late picture presented a few large droplets with vitamin A fluorescence in the Kupffer cells and a few fine droplets at the edges of the adjacent liver cells. If larger fat droplets were present in the cytoplasm of the liver cells, they retained some vitamin A fluorescence for a longer time.

In complete deficiency no fats were demonstrable in the liver with phosphin 3R. In moderate deficiency a reduced fat content was visible, though vitamin A was not necessarily present. The depletion of fat that was demonstrable by phosphin 3R occurred after the depletion of vitamin A. The distribution of the fat at different stages of vitamin A deficiency resembled, tardily, the histologic stages of the loss of vitamin A in the liver. First the fat in the periphery of the lobules was lost, and gradually the process continued centripetally until the fat around the central vein was lost. The liver cell portions adjacent to the fat-containing Kupffer cells retained the fat longer, just as they kept vitamin A longer.

The bulk of vitamin A fluorescence in the kidney, present in the medulla, was lost quickly in depletion, whereas the vitamin A in the endothelial cells of the cortical capillaries remained much longer.

In the adrenal the vitamin A was lost first at the inner and outer margin of the fascicular layer. The last fluorescence was seen in the center.

The adrenal in the growing rat was depleted of vitamin A relatively early, within about nine days. The interstitium of the renal cortex, however, showed increased amounts of vitamin A at the time when the liver was losing its last stores.

Depletion in Adult Rats.—In adult animals (9 rats) the depletion differed from that in growing rats in the following ways: (*a*) The depletion period was longer, and the animal continued to gain weight for a longer period. Rats which previously had been on a control diet of 300 international units daily for from three to six months lost their last vitamin A stores after more than six months on a vitamin A–free diet. (*b*) The fat stores were still present at the time of depletion. (*c*) The accumulation of vitamin A in the interstitium of the renal cortex was not seen in the adult rat at the time when the liver was losing its stores. (*d*) The adrenal cortex of the adult rat contained high amounts of vitamin A at a time when the liver, kidney and lung were practically free of vitamin A.

Replacement by Oral Administration of Vitamin A.—If vitamin A dissolved in oil was given (51 rats) in sufficient quantity, it appeared, except for the intestine, first in the Kupffer cells and in small droplets

in the adjacent parts of the liver cells. The Kupffer cells which were first filled were seen around the central veins. Then the remaining Kupffer cells were filled, and fluorescing droplets appeared lining the entire edge of each liver cell. Seemingly the appearance of fat took place before the restoration of vitamin A.

In the kidney vitamin A was first seen in the interstitium of the cortex. Later the lipoid depots of the medulla imparted the fluorescence.

In the adrenal it was first seen in the midzone of the fascicular layer, seemingly after the restoration of the lipoids.

Vitamin A fluorescence reappeared in the livers of deficient rats (12 rats) after administration of more than 200 international units of vitamin A. The rapidity of reappearance depended on the amount fed. In 1 case, after 6,000 international units had been fed, the fluorescence was seen after two and a half hours. Usually, if 1,000 to 6,000 units had been fed (17 rats), the vitamin A fluorescence reappeared in four and a half hours. With the near minimal dose of 250 units, it was visible in the liver fifteen hours after feeding; the maximal fluorescence was seen after between twenty-five and forty-eight hours.

The vitamin A fluorescence was restored in the kidney much later than in the liver. The adrenal replacement picture paralleled that of the liver; that of the lung paralleled that of the kidney. The replacement in the ovary required a higher total dose.

The intestine showed vitamin A fluorescence at the time of absorption. If more than 900 international units of vitamin A was fed, the vitamin A fluorescence was usually found in the intestinal wall two hours later (16 rats). After a longer interval—upper limit was twenty-six hours—no vitamin A fluorescence was found in the intestinal wall.

Figure 3 shows the fate of a single dose of vitamin A fed to a rat deficient in vitamin A. A dose of 250 international units, near minimal for histologic visualization, maintained a histologically visible storage in the rat liver for about ten days. Although the liver no longer showed the fluorescence after this time, the other vitamin A–containing organs still retained relatively high amounts. There was an accumulation of vitamin A in the cortical interstitium of the kidneys, as was seen in the depleted growing rat when the liver was losing its vitamin A store. From the quantitative point of view the store in the liver was by far the greatest. Thus, the accumulation in the other organs was not significant for the total vitamin A content of the animal.

Replacement by Oral Administration of Carotene.—The smallest amount of carotene required to produce vitamin A fluorescence in the depleted rat was equivalent to the minimal dose of vitamin A (200 to 300 international units). The results in 67 rats, however, were variable, and the fluorescence appeared later and in smaller amounts. A

dose of carotene equivalent to 2,000 international units of vitamin A produced regularly the vitamin A fluorescence in the various organs at the optimal time (twenty-five hours).

There were variations from animal to animal in the initial vitamin A fluorescence seen in the organs after feeding carotene. In some cases (figs. 4 and 5) the first vitamin A fluorescence was seen in the cells in the lamina propria of the villi of the upper part of the small intestine. A slowly fading faint green fluorescence was noted in the epithelium and in the lumen in 10 of 62 rats examined. This may be attributed to the presence of carotene. At times the first vitamin A fluorescence

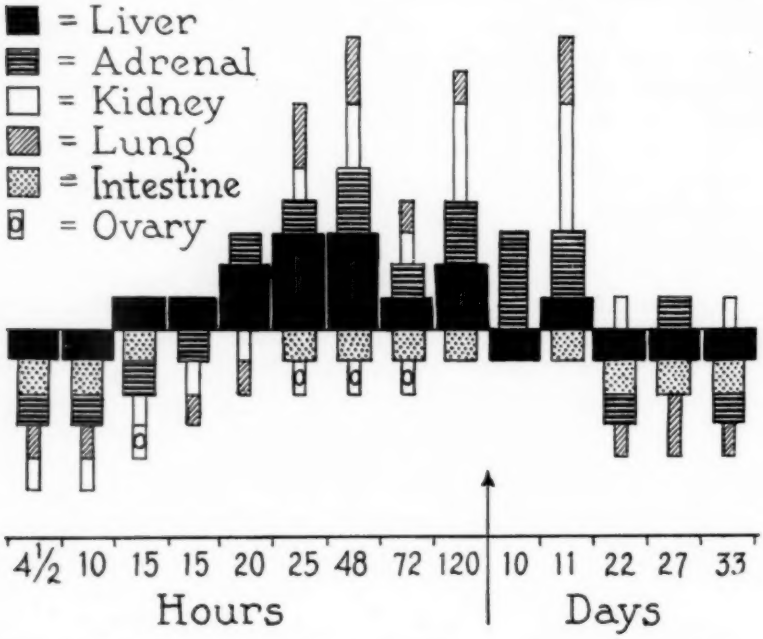

Fig. 3.—Estimation of the vitamin A fluorescence in the organs of vitamin A–deficient rats after the rats had been fed 250 international units of vitamin A (0.25 cc.). Each column represents a rat. The quantity of vitamin A fluorescence is symbolized by both the height and the width of the column. The bars below the base line indicate absence of vitamin A fluorescence.

appeared in the liver. Prior to this the liver contained a fainter green, slowly fading fluorescence. This too was probably due to the presence of carotene. The latter grade of fluorescence was spread over the liver cells and seemingly accumulated at the edges of the liver cells in lipoid droplets, which could be demonstrated by phosphin 3R staining. Since the carotene fluorescence was not distinct, a definite localization was not attempted. A histologic demonstration of carotene in the form of

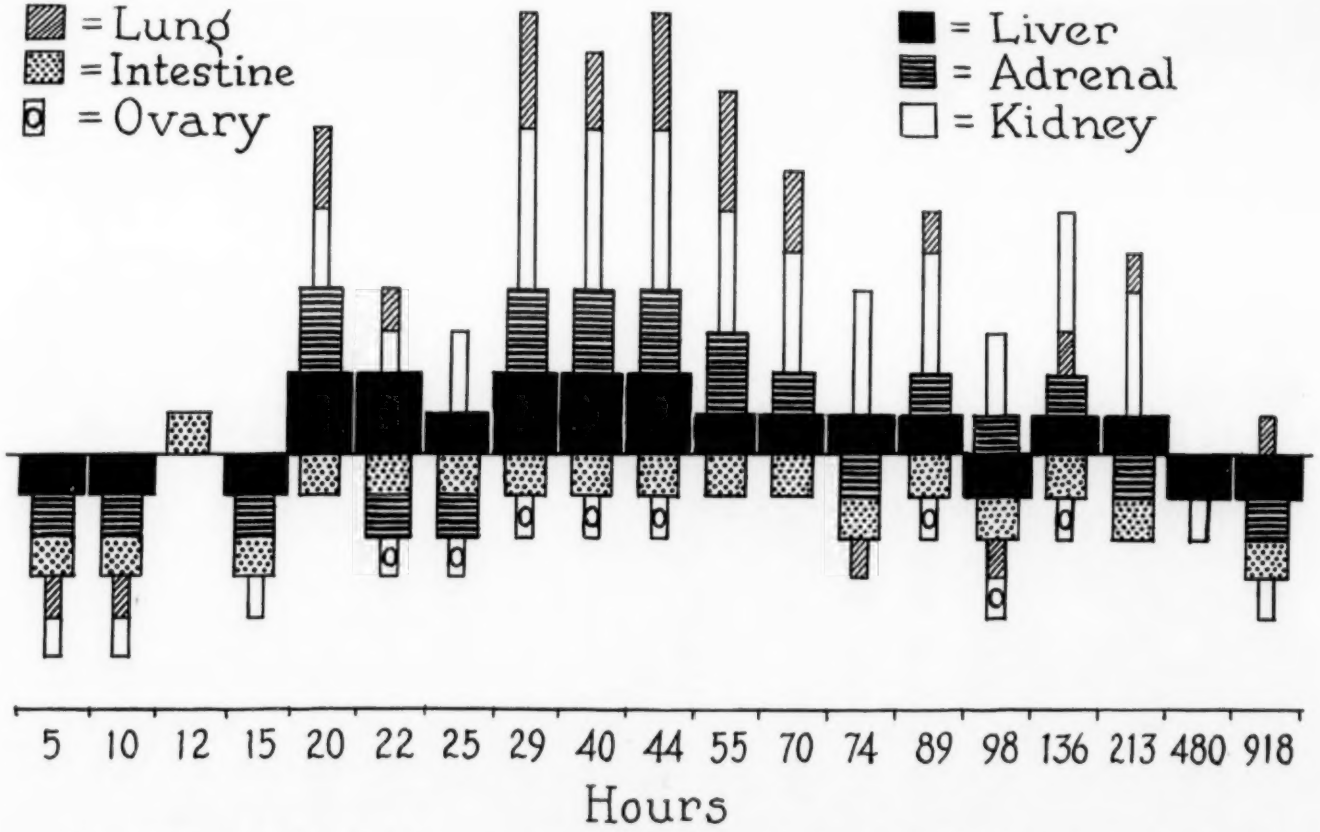

Fig. 4.—Estimation of the vitamin A fluorescence in the organs of vitamin A–deficient rats after the rats had been fed carotene equivalent to 1,000 international units of vitamin A (0.10 cc.). See figure 3.

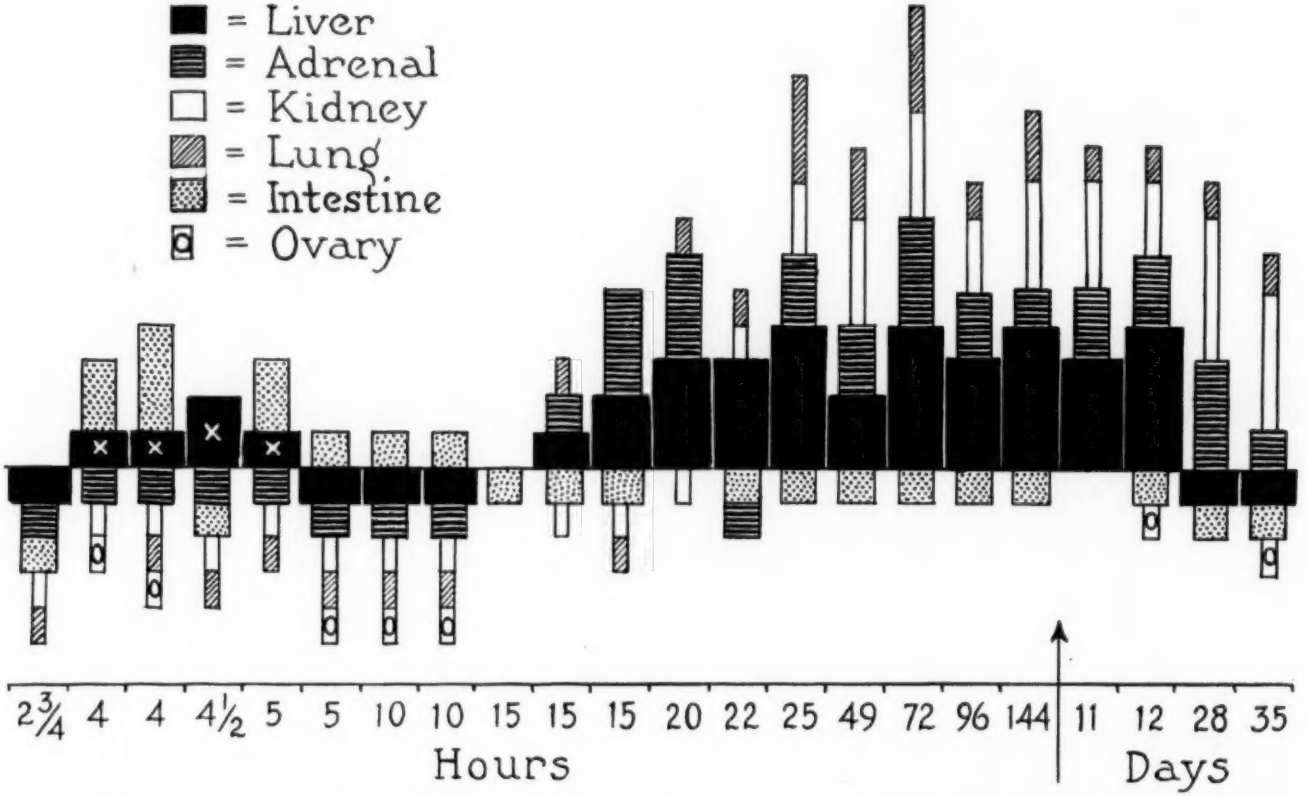

Fig. 5.—Estimation of the vitamin A fluorescence in the organs of vitamin A–deficient rats after the animals had been fed carotene equivalent to 5,000 international units of vitamin A (0.50 cc.). See figure 3. In the liver, bars with crosses, the fluorescence is due to carotene.

pigment inclusions in the Kupffer cells had been reported,[23] and the presence of carotene has been shown by chemical analyses. After carotene feeding vitamin A fluorescence was first visible in the Kupffer cells and then in fine droplets in the adjacent parts of the liver cells, localized around the central veins. The histologic picture of carotene replacement was similar to that of replacement of vitamin A.

In several cases the interstitium of the renal cortex or the fascicular layer of the adrenal showed vitamin A fluorescence prior to the appearance of the latter in the liver after oral replacement with carotene. At the same time it may appear in the interstitium of the lung.

In figures 4 and 5 the fate of a single dose of carotene fed to rats deficient in vitamin A is illustrated. After the equivalent of 1,000 international units of vitamin A (fig. 4) had been fed, the characteristic fluorescence first appeared in the intestine at twelve hours. By the twentieth hour, the intestine was free of vitamin A, but the other organs still retained their vitamin A in unchanged amounts. After twenty days practically all the organs had lost their vitamin A. The histologic evidence of depletion after carotene feeding was similar to that in the growing animals, since the carotene had been changed to vitamin A.

After 5,000 international units of carotene (vitamin A equivalent) (fig. 5) had been fed, the depletion time was prolonged; the disappearance of the fluorescence was not completed by the thirty-fifth day. Maximum storage was also prolonged and was seen from the twentieth hour to the twelfth day after feeding. Five hours after the administration of carotene the liver showed a faint, slowly fading green fluorescence, which was probably due to carotene. The intestine showed vitamin A fluorescence from the fourth to the fifteenth hour after feeding. By the fifteenth hour vitamin A fluorescence was noted in the other organs.

Replacement by Parenteral Routes.—The replacement of vitamin A fluorescence after subcutaneous and intraperitoneal administration of vitamin A or carotene to 18 depleted rats differed in several ways from replacement by the oral route.[24] The time was much prolonged. At least eighteen hours was required after the injection of 5,000 international units of vitamin A (12 rats); at times no evidence of the typical fluorescence was found after a much longer interval. The intestine did not show the fluorescence, which appeared first in the kidney and adrenal and then in the liver. Carotene, administered parenterally, did not in our experiments restore vitamin A fluorescence to the organs.

23. Drummond, J. C.; Gilding, H. P., and MacWalter, R. J.: J. Physiol. **82**:75, 1934.

24. With, T. K.: Nord. med. (Hospitalstid.) **3**:2906, 1939.

carotene equivalent to 5,000 international units of vitamin A (0.50 cc.). See figure 3. In the liver, bars with crosses, the fluorescence is due to carotene.

COMMENT

The histologic demonstration of the distribution of vitamin A in rats by means of fluorescence microscopy confirms the results obtained by chemical and biologic methods. There is no agreement between the pictures obtained by fluorescence microscopy and those obtained by the histologic application of the Carr-Price reaction.[5] Our findings are in agreement with those from the fluorescence microscopic examinations of Schairer and co-workers[4b] on the liver and with those of Jancsó and Jancsó on the eye. They are partly in agreement with the vital microscopic studies of Hirt and Wimmer.[3b] Only a part of the histologic findings of Querner[3a] have been confirmed. The greatest amount of vitamin A fluorescence is in the liver, which chemically contains about 95 per cent of the vitamin stored in the body.[25] The other organs contain much smaller quantities, depending on the state of the animal. There is no characteristic fluorescence in the specific parenchymatous tissue in the pancreas, spleen, thyroid, heart and brain, which, according to the analyses made by McCoord and Luce-Clausen,[1c] contain less than 0.3 international unit of vitamin A. Probably only the blood or the fat cells of these tissues contain vitamin A. Other organ systems of greater mass, such as muscle, skin, bone, intestine or blood, which chemically reveal somewhat higher amounts, do not show vitamin A fluorescence. In the liver, kidney, adrenal and lung, which contain more than 0.7 international unit of vitamin A, according to McCoord and Luce-Clausen,[1c] the fluorescence is noted in the parenchyma.

The parallel results obtained chemically by others and by us, and histologically by us and also by Schairer and co-workers,[4b] in deficiency, in normality and in hypervitaminosis are to be considered as a strong basis for the validity of the histologic technic. Decisive evidence is offered by replacement experiments. Known amounts of vitamin A were fed to vitamin A–deficient animals whose livers did not show vitamin A fluorescence. The resulting fluorescence of the liver agreed with the amount of vitamin A fed and with the amount determined chemically. With the exception of vitamin A and carotene, we did not come across a single substance which produced the green fading fluorescence in the liver of the deficient rat.

It appears that the only point of evidence required to establish absolutely that the fluorescence referred to is vitamin A fluorescence is to show that the wavelength of crystalline vitamin A is identical with that of the green fluorescence fading under ultraviolet rays in the liver.

25. Sherman, A. C., and Boynton, L. C.: J. Am. Chem. Soc. **47**:1646, 1925. Moore.[1a]

The rate of restoration of the vitamin A fluorescence to the organs depends on the amount of the vitamin fed.[26] With vitamin A, regular results were obtained if the deficient animals used were still gaining weight. At this early stage of vitamin A deficiency the intestinal absorption was reproducible. The lowest dose of vitamin A which produced definite vitamin A fluorescence in the liver twenty hours after feeding was 250 international units. The minimal dose and the minimum time required for restoration of the fluorescence agreed roughly with the findings of Bauman, Riising and Steenbock.[11b] They maintained, on the basis of chemical analyses, that a higher amount of vitamin A is necessary to produce storage than to promote growth in deficient rats. With low doses of carotene, the production of vitamin A fluorescence in deficient animals was not as regular as with vitamin A. Likewise, chemical analysis showed the irregularity of the absorption of carotene and the need for higher doses.[27] A period of several hours is required for the transformation of carotene to vitamin A.

The facts stated should be the basis for the use of this technic in assays for the vitamin A and carotene contents of oils. Intestinal absorption is not likely to be as regular in technics which require a deficiency sufficient to produce clinical symptoms. The time required for the assay is less.

The delay in the restoration after parenteral administration is due to the faulty absorption of fatty substances in the subcutaneous tissue. The reason for the retarded appearance of vitamin A fluorescence in the liver after this kind of administration is not clear. The state of the vitamin A (free or combined) is probably a factor.[28]

The route that vitamin A follows after it has been administered to the deficient animal can be studied by the histologic method. The path of the vitamin through the intestinal wall and the lymphatic transportation of the vitamin from the intestine are quite evident (also shown by Drummond, Bell and Palmer [27a] in a patient with chylothorax).

In the liver we found vitamin A fluorescence after a lapse of time which allowed for the passage of vitamin A through the intestine and from the intestine to the liver. Vitamin A fluorescence was seen in the liver as early as four hours after the feeding of the optimal dose. These findings, supported by chemical analysis of the same liver, are in con-

26. Steenbock, H.; Sell, M. T., and Nelson, E. M.: J. Biol. Chem. **56**:327, 1923.

27. (*a*) Drummond, J. C.; Bell, M. E., and Palmer, E. T.: Brit. M. J. **1**:1208, 1935. (*b*) Heyman, W.: Am. J. Dis. Child. **51**:273, 1936. (*c*) Howard, K. H.: Biochem. J. **30**:1878, 1936. (*d*) Ahmad, B.: ibid. **25**:1195, 1931. (*e*) Clausen.[20]

28. (*a*) Emmett, A. C., and Bird, O. D.: J. Biol. Chem. **119**:31, 1937. (*b*) Gray, E. L.; Hickman, K. C. D., and Brown, E. F.: J. Nutrition **19**:39, 1940. (*c*) Drummond and others.[27a]

trast to the report of With,[29] who found it in the liver by chemical means within one hour after feeding vitamin A and two hours after feeding carotene. The histologic studies suggest that fat is replaced prior to vitamin A.

In the liver vitamin A fluorescence appears first in the Kupffer cells and in the adjacent parts of the liver cells. Seemingly, the Kupffer cells receive vitamin A from the blood and immediately transmit some of it to the liver cells. Jaffé and Berman [30] showed that after an intravenous injection of a fat preparation the transmission to the liver cells from the Kupffer cells starts shortly after the injection. Concomitantly with, or shortly after its appearance in the liver, vitamin A fluorescence is seen in the epithelial cells of the adrenal cortex and in the interstitium of the renal cortex and lung, where it is apparently in the endothelial cells of the capillaries. This parallel behavior of the endothelial cells of the liver, kidney and lung is interesting, especially since no vitamin A fluorescence is seen in the endothelial cells of the spleen.

The role of the endothelial cells of the peritubular capillaries in the renal cortex should be especially noted. Here vitamin A is not bound to demonstrable lipoids; its distribution follows certain nephrons, and the cells show vitamin A fluorescence after replacement long before the other vitamin A storage sites of the kidney. Finally, the fluorescence is increased in amount during depletion of the growing animal. In our review of the literature we did not find reference to any special role played by the endothelial cells of the peritubular capillaries (except that they show high fat storage in hypervitaminosis A).[9d] The nephron-bound distribution points to an intermittent activity of the renal glomeruli. Possibly, in the tubular capillaries connected with functioning glomeruli the concentration of vitamin A is increased as a result of the glomerular filtration and reabsorption of fluid.

The transformation of carotene to vitamin A was assumed to occur chiefly in the liver cells. These were considered to be the functioning cells [31] because the conversion is decreased in phosphorus poisoning [32] and because in certain types of parenchymatous damage in cattle retention of carotene is shown in the liver cells.[33] The histologic evidence suggests several sites for the transformation of carotene to vitamin A. The first fluorescence after the feeding of carotene was seen in the intestine or in the Kupffer cells and then in the adjacent parts of the liver cells or in the endothelial cells of the renal cortex and lung or

29. With, T. K.: Nord. med. (Hospitalstid.) **3**:2901, 1939.

30. Jaffé, R. H., and Berman, S. L.: Arch. Path. **5**:1020, 1928.

31. Olcott, H. S., and McCann, D. C.: J. Biol. Chem. **94**:185, 1931. Moore.[1a]

32. Graeves, J. D., and Schmidt, C. L. A.: Am. J. Physiol. **111**:502, 1935.

33. Buckley, J. S.; Joss, E. C.; Creech, G. T., and Couch, J. F.: J. Agric. Research **40**:991, 1930.

in the adrenal cortex. Although storage of carotene takes place in the liver cells, probably in their lipoids, there is no histologic evidence showing that the liver cells are the only sites for the transformation.

The significance of the Kupffer cells in vitamin A metabolism has been pointed out.[3b] Reduced vitamin A storage in the liver after blockage of the reticuloendothelial system has been described.[34] Our examinations suggest that the Kupffer cells of the liver, abetted by the endothelial cells of the kidney and lung, are the first to store vitamin A during replacement. They are the last to lose it in depletion. They are probably also concerned with the splitting of carotene.

The excess of the vitamin A in hypervitaminosis is carried in the Kupffer cells. The rat quickly loses the excess in storage during hypervitaminosis if the vitamin is withheld. The depletion time is supposedly the same for well fed normal rats as for hypervitaminotic rats.[12] Apparently, the Kupffer cells lose their excess of vitamin A rather quickly; probably the liver cell is the important permanent storage place for vitamin A.

The histologic distribution of vitamin A in the rat is similar to that in man, as shown by one of us.[8] In a review of the distribution of vitamin A, the vitamin was found in demonstrable amounts not only in the liver cells, but also in the eye, apparently connected with vision (Wald cycle); in the adrenal and ovary, probably connected with the presence of fat-soluble hormones; in the endothelial cells of different organs, and in the fat cells, where it is stored. The vitamin A in the larger fat droplets is possibly less available for immediate use.

Histologically, vitamin A was not found in the lining epitheliums (e. g., cornea, bronchial epithelium, renal pelvis, gastrointestinal tract except during absorption of vitamin A). It was not seen in the enamel organ, which was intact in freshly prepared ground sections (examinations with M. M. Hoffman of the department of histology of the University of Illinois School of Dentistry). These structures are the first to be involved clinically in vitamin A deficiency.[35]

SUMMARY

The organs of rats were examined for vitamin A by fluorescence microscopy. The following evidence is presented in support of the concept that the green fading fluorescence in tissues is due to vitamin A: (*a*) Chemical analysis for vitamin A confirmed the histologic observations. (*b*) The amount of the fluorescence produced in vitamin

34. Lasch, F., and Roller, D.: Klin. Wchnschr. **15**:1636, 1936. Wendt, H., and Koenig, D.: ibid. **16**:1253, 1937. Uotila, U., and Simolas, P. E.: Virchows Arch. f. path. Anat. **301**:523, 1938.

35. Wolbach, S. B.: J. A. M. A. **108**:7, 1937.

A–deficient rats by replacement with vitamin A was correlated with the amount fed and with the amount determined chemically in the same liver. (*c*) The feeding of substances other than vitamin A or carotene did not produce the fluorescence in the deficient rats. The only point required to establish the concept that the fluorescence is actually due to vitamin A is the determination of the identity of the wavelength of the fluorescence of the liver and of crystalline vitamin A.

The characteristic fluorescence was found in the epithelial and Kupffer cells of the liver, the fascicular layer of the adrenal, the interstitium of the kidney and lung, the interstitial cell cords and the corpora lutea of the ovary, in the pleura, pericardium and peritoneum, in the meninges, in the retina and pigment layer of the eye, in fat cells, and in the upper part of the small intestine during absorption of vitamin A.

In hypervitaminosis A all the locations mentioned, especially the Kupffer cells, showed much more vitamin A fluorescence.

In newborn rats traces, in young animals small amounts, of the vitamin A fluorescence were found.

The histologic picture in depletion and that in restoration of the vitamin A depots have been described. Even in severe deficiency the light-adapted eye retained some vitamin A. The depletion of young rats was completed (except for the retina) within fifteen days. This permitted reliable replacement studies and served as the basis of a possible technic for assaying the vitamin A contents of oils. Restoration by subcutaneous administration required a longer time than that by ingestion. A lymphatic route for absorption was indicated.

Restoration of vitamin A fluorescence by feeding deficient animals carotene was less regular and required more time. The first fluorescence appeared in the lamina propria of the intestine, the Kupffer cells, the adrenal cortex and the endothelial cells of the renal cortex and of the lung. Probably carotene is converted into vitamin A in one or all of these locations.

SO-CALLED INTERCAPILLARY GLOMERULOSCLEROSIS—A LESION ASSOCIATED WITH DIABETES MELLITUS

MORPHOGENESIS AND SIGNIFICANCE

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In 1936 Kimmelstiel and Wilson [1] published a report of 8 cases in which they demonstrated the association of a peculiar lesion of the kidney with a clinical syndrome. The renal lesion was characterized by a type of focal glomerular hyalinization, which they called "intercapillary glomerulosclerosis." The clinical complex consisted typically of diabetes, hypertension and the nephrotic syndrome. During the same year Murakami [2] observed the lesion in a case of diabetes manifesting an incomplete syndrome. Two years later Anson [3] and more recently Derow, Altschule and Schlesinger,[4] and Newburger and Peters [5] confirmed the association. The latter reports have been concerned primarily with elaborations of the clinical aspect of the problem. On the part of pathologists there appears to have been relative indifference toward any clinicopathologic correlation. This may be accounted for as follows: First, the lesion, which has been known to pathologists for a long time, is regarded as representing simply an advanced stage of the glomerular atrophy of ordinary nephrosclerosis; secondly, Kimmelstiel and Wilson have tended to reduce the issue to mere academic importance by their statement that the lesion is of infrequent occurrence. It is the purpose of this paper to determine the validity of these presumptions, and, in addition, to try to answer certain reasonable key questions, such as:

1. Does the lesion occur only in patients with diabetes?

2. What is the correlation between the extent of the lesion and the severity of the clinical picture?

From the Laboratories of the Mount Sinai Hospital.

1. Kimmelstiel, P., and Wilson, C.: Am. J. Path. **12**:83, 1936.
2. Murakami, R.: Tr. Soc. path. jap. **26**:657, 1936.
3. Anson, L. J.: South. M. J. **31**:1272, 1938.
4. Derow, H. A.; Altschule, M. D., and Schlesinger, M. J.: New England J. Med. **221**:1012, 1939.
5. Newburger, R. A., and Peters, J. P.: Arch. Int. Med. **64**:1252, 1939.
6. Reference deleted by the author.

3. What is the morphogenesis of the lesion and what is its role in the causation of the abnormal clinical physiology?

4. Is there anything peculiar about the pancreas in these cases?

The clinical correlation has been considered in a separate communication (Siegal and Allen [7]). This paper will deal principally with the morphology of the glomeruli in diabetes and the interpretation thereof.

MATERIAL

The kidneys studied were taken from 105 consecutive diabetic patients over the age of 40, 100 consecutive patients above 40 years of age with blood pressure 160 systolic and 90 diastolic or greater, 100 consecutive nondiabetic, nonhypertensive controls and 34 unselected patients with subacute and chronic glomerulonephritis.

METHODS

The tissues were fixed routinely in solution of formaldehyde U. S. P. diluted 1:5. Zenker-formaldehyde solution (20:1) [8] and absolute alcohol were used occasionally. Hematoxylin and eosin, Mallory-Heidenhain azocarmine,[9] Mallory's phosphotungstic acid-hematoxylin, Masson's trichrome (Goldner's modification),[10] a modified Bielschowsky silver stain, Best's carmine, crystal violet (amyloid) and sudan stains were used. The interpretation of the sections stained with azocarmine was made only on tissue fixed in Zenker-formaldehyde solution. Representative sections were digested with trypsin, usually for twenty-four hours, after which they were stained with azocarmine or the Van Gieson stain. The lesions of 6 kidneys were studied in sections cut serially at 3 and 6 microns.

Since nearly every one of the stains contributed an appreciable and well defined portion of the picture, the stains will be considered separately and the observations will be finally integrated into a unified concept of the lesion. The attempt will be made to clarify this concept further by contrasting the glomerular changes in diabetes with those observed in nondiabetic nephrosclerosis and in glomerulonephritis. The lesions will henceforth be referred to as the "diabetic lesions" for convenience.

HEMATOXYLIN AND EOSIN

General Features.—The lesions in the kidneys may be diffuse or sparse. They may involve practically all of the glomeruli in a section or merely one, and this isolated lesion may be quite as typical as those in the more extensively affected kidneys. They characteristically show much variability in size, ranging from approximately 20 microns to about 120 microns in their greatest diameter. Hence, a large lesion may occupy more than a third of the volume of a glomerulus. The lesions are generally more or less spherical, although occasional oval ones are

7. Siegal, S., and Allen, A. C.: Am. J. M. Sc. **201**:516, 1941.

8. This is Zenker's solution prepared with solution of formaldehyde instead of glacial acetic acid.

9. The Mallory-Heidenhain method was slightly modified by replacing acetic acid in the aniline blue–orange G solution with 2 per cent oxalic acid and by mordanting in phosphotungstic acid for only twenty minutes.

10. Goldner, J.: Am. J. Path. **14**:237, 1938.

seen, with the longer diameter two to three times the other. The involved glomeruli, in spite of the hyalinization, need not be small; on the contrary, they may be as large or even frequently larger than normal. In some glomeruli the hyaline mass may focally bulge the portion of Bowman's capsule immediately about it. Bowman's space may be of normal width; not infrequently, however, it is widened and may be filled with protein precipitate.

The typical lesion consists of a hyalinized acidophilic material, which with low magnification appears almost completely homogeneous. With greater magnification, one can make out a circumferentially laminated structure, often pitted with a few small vacuoles. The fibrillated hyalin is dull, manifesting little or none of the refractility of the "wire loops" seen in the glomeruli involved in acute disseminated lupus erythematosus. The acidophilia of the lesions, as a rule, is not quite as marked as that of the walls of the arterioles. The hyalin does not react positively with stains for amyloid.

Cellularity.—The cellularity of the lesions varies in quantity, disposition and character. Often there are two to three concentric layers of flattened cells. These appear to be displaced endothelial and epithelial cells. The core is generally devoid of cells, although occasional vesicular or more or less pyknotic, distorted nuclei, and even a red blood cell or two, may be found near the center. One may not infrequently acquire the impression that in some of the lesions there is an increase of mononuclear cells. This appears to result from cutting the lesion in a plane passing through the cellular rim at the periphery of the lesion (fig. 2). Here and there, endothelial and epithelial foam cells are seen. These have been observed previously in kidneys from patients with diabetes by Murakami [2] and in kidneys from patient's with nephrosis and from patients with Niemann-Pick disease by Kantrowitz and Klemperer.[11]

Serial Sections.—Reconstruction of the lesion from serial sections reveals that it is not a bar of hyalin which extends from the hilar arterioles to the periphery of the glomerulus, as Kimmelstiel and Wilson [1] appear to have believed. Rather, it seems to be a mass of hyalin isolated to a single or to adjacent lobules and formed by an abrupt profound thickening of the wall of one or more closely situated capillaries, with resultant encroachment on and obliteration of their lumens (fig. 1). These lumens are almost invariably obliterated in sections through the "equator" of the mass or through its great arcs, but near the poles of the globes, i. e., as one approaches tangential sections, lumens containing red blood cells and lined by endothelium frequently become apparent (fig. 1). In kidneys with widespread lesions, one may observe in a single section of a glomerulus the crude equivalent of serial sections in the form of a variety of transition phases of the capillary loops, although in reality they belong to multiple lesions. In such glomeruli one finds normal capillary loops, loops of which only a crescentic segment has undergone the characteristic hyalinization, loops whose entire wall has become hyalinized so as to leave only a narrow lumen containing one or two red blood cells and, finally, the typical hyalin disk in which the lumen has been obliterated. The larger disks result from the coalescence of multiple capillary loops.

Peripheral Capillary Loops.—A characteristic and physiologically probably important component of the lesion consists of the capillaries situated at the rim of the lesion. Immediately external to the hyaline mass, the capillary walls not yet

11. Kantrowitz, A. R., and Klemperer, P.: Virchows Arch. f. path. Anat. **280**:554, 1931.

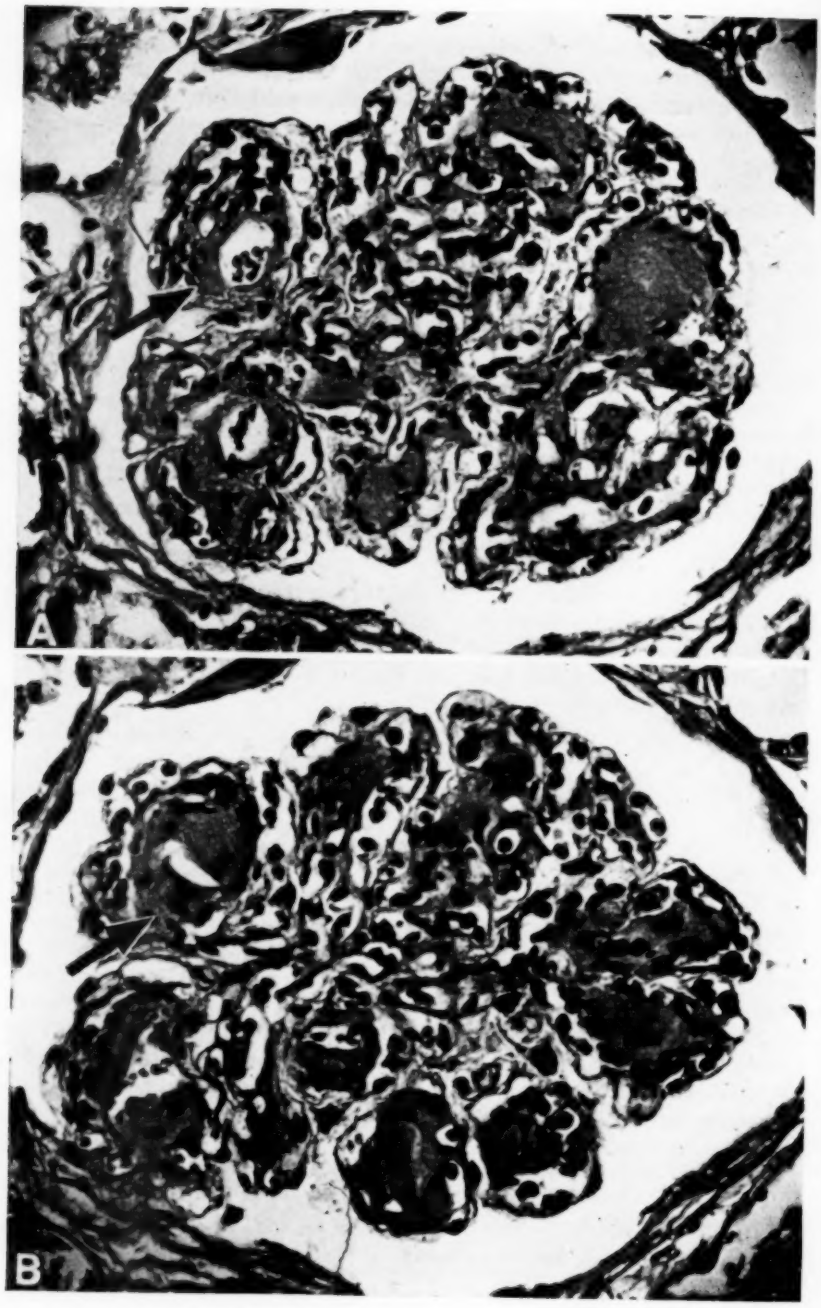

Figure 1

(See legend on opposite page)

a part of the lesion are thickened and their lumens are partially to completely compressed. However, one or more of the most peripheral capillaries are practically constantly dilated at some level of their course as observed in serial sections. Commonly, one sees a single loop partially or completely circumscribed about a large diabetic lesion, reminding one somewhat of a fertilized ovum of Ascaris. Even though the space between the centrally placed disk and the external wall of such capillaries may appear deceptively small, nevertheless it is obvious that these capillaries, encircling, as they do, the large hyaline mass, are greatly dilated. These peripheral capillaries may be jammed solid with red blood cells, or they may contain laked blood and much protein precipitate, some of which may have escaped into Bowman's space (fig. 7). Some of the most strikingly dilated loops actually contain small clots composed of a delicate fibrin mesh and platelets. The walls of these distended capillaries have escaped thickening and often appear to have been stretched excessively. The possible physiologic significance of this finding will be discussed later.

Of course, in the same section there are, in addition, glomeruli which exhibit the changes characteristic of ordinary nephrosclerosis. As a matter of fact, these changes may be present in the very same glomeruli involved by the diabetic lesion and be easily distinguishable from the latter. The simple nephrosclerotic portions of the glomeruli are differentiated by several criteria, chief among which is the feature that no one capillary loop appreciably outstrips its neighbor in degree of hyalinization. This holds even in the most advanced cases of nephrosclerosis and is in contrast to the typically focal hyalinization in the diabetic kidney (fig. 3).

MALLORY-HEIDENHAIN AZOCARMINE

With the azan stain, the lesions manifest three different tinctorial qualities:

1. A homogeneous dark blue with evidence of fibrillation, quite like mature collagen. This is the most characteristic reaction.
2. Red or pink. This coloration occurs infrequently and in small amounts.
3. Purple-red or purple-orange. This appears to represent an intermediate transitional phase in the progressive stages of collagenization from the red or pink to the deep blue of mature collagen.

In following the development of the lesions in serial sections in order to locate the exact site of the hyaline material, one may observe in the early lesions the basement membrane of the capillaries split or frayed into several layers, which

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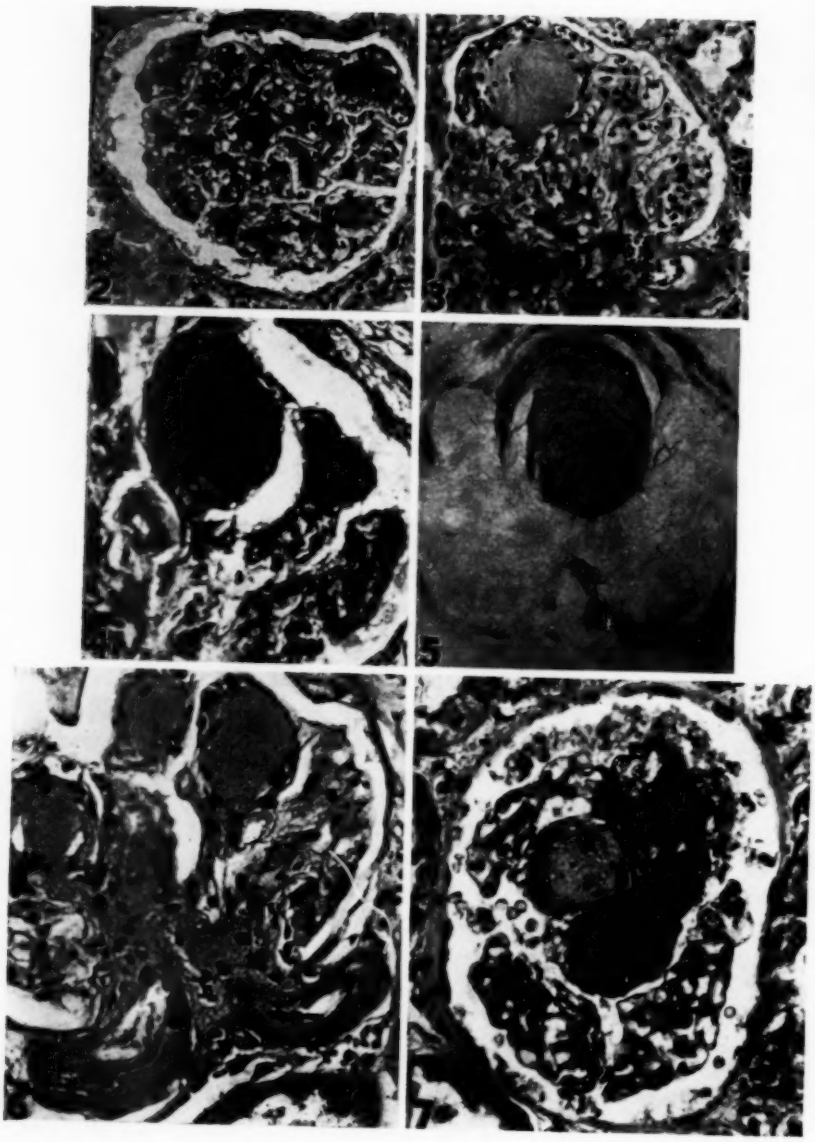
EXPLANATION OF FIGURE 1

This figure shows representative sections of a series. The interval between these two sections is 18 microns. Note in *A* the thickened capillary wall surrounding a lumen containing red blood cells (arrow). Note, too, the adjacent compressed capillaries, which are the source of the crowded endothelial and epithelial cells at the periphery of the incompletely formed diabetic lesion.

In *B* the lesion (arrow) is seen in a more advanced stage of its development, with the lumen almost completely obliterated by the hyalinized capillary wall. The lesion enlarges primarily by progressive sclerosis of the capillary wall and fusion of adjacent thickened capillaries rather than by thickening of the intercapillary tissue. Hematoxylin and eosin; high power.



Figures 2 to 7

(See legend on opposite page)

then become fused into a hyaline mass. Frequently one sees a faintly bluish granular material between the reduplicated basement membrane, as if this were a precursor of the hyalin. Occasionally thick crescents of hyalin are found, which appear to be accretions of collagenous material located unmistakably on the inner (luminal) side of the capillary basement membrane as judged by the presence of red blood cells within the lumen. This position of the hyalin on the inner side of the capillary basement membrane is regarded as an important bit of evidence favoring a mural rather than an intercapillary origin.

Of great interest is the alteration in the collagenous tissue of other portions of the nephron. This has been referred to previously, e. g., by Murakami.[2] A segment of the parietal layer of Bowman's capsule may manifest a fusiform swelling which has partially lost its affinity for aniline blue, and, instead, is stained pink or orange, as in portions of the diabetic lesions. Similar abrupt fusiform thickenings and altered tinctorial reactions may be seen in the basement membranes of the convoluted tubules. In each of these situations, as well as in the arterioles, there generally persists a fine blue line at the periphery of the altered material as if the outer rim had escaped the alteration. Qualitatively similar but less marked changes may be observed in the nephrosclerotic kidneys of nondiabetic persons.

Mallory's phosphotungstic acid-hematoxylin and the modified Masson stain give results which are directly analogous to those seen with the azocarmine stain and so need not be described.

SILVER STAIN

The silver stain is of particular help in the analysis of the lesion. Characteristically, the diabetic hyaline body which stains a more or less homogeneous pink

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Explanation of Figures 2 to 7

Fig. 2.—Note the focal collection of cells at the upper right in the glomerulus. This deceptive appearance of cellular proliferation is the result of a lesion having been sectioned in a plane passing through its rim of displaced and crowded endothelial and epithelial cells. Hematoxylin and eosin; medium power.

Fig. 3.—Note that the hyaline diabetic lesion (upper left) is at a pole directly opposite that of the hilus and that there is no hyaline continuity between the two. Note, too, the great disparity between the marked focal hyalinization in one portion of the glomerulus and the normal capillary loops in others. Hematoxylin and eosin; medium power.

Fig. 4.—Note the intense and characteristically laminated argyrophilia of the diabetic lesion. Modified Bielschowsky stain; high power.

Fig. 5.—This photomicrograph illustrates the resistance of the diabetic lesion to tryptic digestion as contrasted with the remainder of the glomerulus and the afferent arteriole. Tryptic digestion followed by Mallory-Heidenhain stain; high power.

Fig. 6.—Note the thickened afferent and efferent (right) arterioles in a glomerulus with three diabetic lesions. Hematoxylin and eosin; high power.

Fig. 7.—Photomicrograph illustrating a diabetic lesion adjacent to a markedly dilated loop jammed with red blood cells. Note the protein precipitate, as well as a few red blood cells, in Bowman's space. Masson's stain (Goldner's modification); high power.

with hematoxylin and eosin, is found to be composed of a mass of black or deep brown fibers, which are often laminated and are easily and sharply differentiated from the uninvolved portions of the glomerulus. Even the crescentic mural capillary thickenings of the early or small lesions are revealed as laminations of argyrophilic or dense collagenous fibers or as deep brown, clearly distinguished masses. The remarkable feature is the pronounced difference between the argyrophilia of the lesions and the relatively slight affinity for silver, with the Bielschowsky stain, of the remaining capillary loops even within the same glomerulus, however atrophied this organ may be. Even when a fairly complete impregnation of the nonspecific portions of the involved glomerulus is achieved, the delicate, often reticular silver fibers may be unmistakably differentiated from those of the diabetic lesions. Some of the large lesions may show argyrophilic fibers present at the periphery while the core stains the golden or deep brown characteristic of dense collagen. This brown coloration differs distinctly from the pink of the counterstained atrophied capillaries of the nonspecific sclerotic portions of the glomerulus. One does see long wavy collagenous and argyrophilic fibers not infrequently in the crescentically thickened Bowman's capsule. However, these offer no diagnostic difficulty. The hyalin of the diabetic lesion thus becomes distinguishable with a high degree of reliability from the hyalin of the simple nephrosclerotic lesion in the glomerulus. It might be added that in tinctorial properties the hyalin of the diabetic lesion differs too from that found in the ovarian, uterine and splenic vessels and from the hyalin present in the islets of Langerhans.

SUDAN STAIN

There is commonly a moderate amount of fat, neutral chiefly, but some anisotropic, which is located principally in the convoluted tubules and in the arterioles and interlobular arteries. Crescentic deposits of sudanophilic material are found also in Bowman's space. Occasionally one or two sudanophilic cells are seen there. A few fat droplets are dispersed in endothelial and epithelial cells within glomeruli. The diabetic lesion itself may contain no fat or just scattered minute droplets, which in some of the incompletely collagenized ones may collect into larger globules. As Kimmelstiel and Wilson[1] pointed out, one can hardly attach much differential significance to the lipoid deposits inasmuch as they may be present in the nephrosclerotic kidneys of nondiabetic persons and certainly in diabetic kidneys without glomerular lesions.

RESULTS OF TRYPTIC DIGESTION

In the attempt to define further the particular type of hyalin in these kidneys, and to distinguish the hyalin of the diabetic glomerular lesions from that of the arterioles, the digestibility with trypsin was determined. The following are the observations made after digestion by trypsin for an average of twenty-four hours at 37 C. The solution was alkalinized with sodium carbonate and 1 cc. of chloroform was added for its preservative action.

1. The diabetic lesions, as a rule, manifested marked resistance to trypsin. This is in keeping with the frequent marked argyrophilia and dense collagenous composition of the lesions.

2. The remainder of the capillary loops, i. e., those in the nonspecific sclerotic portions of the glomeruli, exhibit slight digestibility. This is demonstrated by the diminished affinity for aniline blue of these loops in the sections exposed to

trypsin, as contrasted with the undigested control slide and with the diabetic lesions in the same section or even in the very same glomerulus (fig. 5).

3. The thickened Bowman capsule and the basement membrane of the tubules resist digestion.

4. A contrast is offered by the digestibility of the hyalinized portions of the arterioles. This pertains principally to the central parts of the arteriolar walls, since the inner and outermost portions are often argyrophilic and hence indigestible.

When one correlates the azocarmine-stained tissues before and after digestion, one observes the following order of digestibility: red or pink, easily digestible; purple-red or purple-orange, moderately digestible; blue, negligibly or slightly digestible.

HYALINE INVOLVEMENT OF ARTERIOLES

There appears to be a practically constant association of the diabetic lesion with afferent arteriolosclerosis. Only rarely are lesions found in glomeruli whose afferent arterioles are thin walled. Occasionally, the arterioles may appear normal in a single section; however, because of the uneven, in places discontinuous thickening that may be present in these vessels, one may find a markedly thickened wall elsewhere along their course if the sections are examined serially.

A finding of considerable interest is the thickening and narrowing of the efferent arterioles by a hyaline material identical with that in the afferent arterioles. The efferent vessels are, as a rule, not as strikingly thickened as the afferent ones, nor are they as constantly affected. For example, in one of the more severely involved kidneys, only about one third of the efferent arterioles are thickened and narrowed as opposed to a practically constant involvement of the afferent vessels.

The hyalin of the arterioles differs in several respects from that of the diabetic glomerular lesions. First, there is usually a much greater abundance of fat in the arteriolar walls. As a matter of fact, the fully collagenized diabetic lesions may contain no fat. Secondly, the arteriolar hyalin is generally more intensely eosinophilic. In addition, one can frequently make out a grayish blue tint in the hyalin of the arterioles stained with hematoxylin and eosin, which is found in the midportions of the wall so as to correspond to a highlight. This tint signifies a "soft" hyalin which is digestible with trypsin and does not take the stains for collagen. Thirdly, the arteriolar hyalin is typically bright red with the azocarmine stain, whereas the diabetic lesions are predominantly blue, indicating the collagenization of the latter. Fourthly, they lack the resistance to tryptic digestion so characteristic of the diabetic lesions (fig. 5). Finally, there is a distinct difference in the amount and disposition of the argyrophilic fibers in the two locations. Similar, though generally less marked changes may be seen in the afferent arterioles of nephrosclerotic kidneys from nondiabetic patients, with the absence, however, of the diabetic glomerular lesions. The prearteriolar interlobular arteries are also frequently thickened and narrowed by irregular hillocks of azocarminophilic hyalinized material.

There may be a remarkable disparity between the degree of vascular involvement, on the one hand, and the relative lack of gross renal contraction and granularity, on the other. This feature was noted by Kimmelstiel and Wilson,[1] who felt that the "contraction may be in part or completely obscured by the signs of nephrosis." What this means in terms of actual histologic change is not clear. Surprisingly enough, it is not a simple matter to account for the illusory relative

gross smoothness and enlargement of the kidneys even from the very sections themselves. To be sure, there usually are numerous areas of cortical atrophy (although this need not be marked even with extensive diabetic glomerular lesions) alternating with foci of dilated tubules, yet without a proportionate granularity and contraction. Failing a micrometric examination, the only morphologic clue one gets is that the scar tissue is not as compact as usual.

SUMMARY OF PRINCIPAL CHARACTERISTICS OF THE LESION

The following, then, are the chief characteristics of the diabetic glomerular lesion:

1. A hyalinized, partially or completely collagenized focal mass frequently found at a distance from the hilus without necessarily having a hyaline continuity with the vascular pole, contrary to Kimmelstiel and Wilson.[1]

2. Variability in size, ranging from approximately 20 to 120 microns.

3. A wide range of glomerular involvement, from only a single glomerulus in a section to practically all of the glomeruli.

4. A peripheral, usually thin-walled ring of capillaries, one or more of which is often markedly dilated and congested, with evidence of stasis.

5. An origin from the capillary walls, including the basement membrane and the surrounding collagenous and reticular mantles when present. This is contrary to the belief of Kimmelstiel and Wilson,[1] who would derive the lesions from a thickening of the so-called axial intercapillary tissue as it extends from the hilus to the periphery.

6. A conspicuously delimited deep brown coloration or a marked argyrophilia (Bielschowsky's stain), especially near the poles or in tangential sections of the lesions. The silver fibers are typically arranged in laminated bands so as to allow differentiation with ease from the glomerular lesion in nondiabetic nephrosclerosis and from the so-called intercapillary lesion of glomerulonephritis.

7. Affinity for aniline blue, with portions of the lesions staining purple-red, pink and purple-orange, thereby suggesting transitional stages in the repair of altered collagen.

8. Relative resistance to tryptic digestion.

9. Association with narrowed afferent as well as narrowed efferent arterioles.

COMMENT

These findings have been set down with considerable detail principally because of the major issues involved and the discrepancies encountered. One of the foremost questions concerns the relationship of the diabetic lesion to the changes observed in the glomeruli of senile degen-

erative (Kimmelstiel [12]) and hypertensive kidneys from nondiabetic persons as well as to those characteristic of intracapillary glomerulonephritis. (The changes within the capillary loops of the glomeruli of senile arteriosclerotic and benign hypertensive kidneys will be considered in the same category for the purpose of this paper; the additional changes seen in malignant nephrosclerosis are not pertinent.)

Kimmelstiel and Wilson [1] expressed the belief that "there is only a difference in degree between the less marked changes frequently observed in senile kidneys" and the diabetic glomerular lesions. Furthermore, they expressed the opinion that the diabetic lesion differs from that found in intracapillary glomerulonephritis only in the alterations found in the basement membrane in this condition. The findings in the present study do not seem to be in accord with these interpretations.

Nephrosclerosis.—Two concomitant processes appear to be concerned in the morphogenesis of the atrophic glomeruli in the nondiabetic nephrosclerotic kidney:

1. Thickening — often crescentic — of Bowman's capsule, with encroachment on the space previously occupied by the capillary loops.

2. Diffuse, more or less regular thickening of the walls of the capillaries with gradual narrowing and obliteration of their lumens and, finally, homogeneous fusion of their walls.

Contrary to this mechanism is that stressed by Kimmelstiel [12] as occurring particularly in the senile arteriosclerotic kidney. He expressed the belief that the compression of the capillaries is produced by diffuse thickening of an axial mesangium—an intercapillary connective tissue.

Neglecting for the time being the issue as to whether or not the increased glomerular collagenous tissue is truly intercapillary, one may nonetheless state categorically that the resultant histologic picture differs in the following clearcut respects from the diabetic lesions:

1. The so-called mesangium is stated to be most marked at the hilus of the glomerulus and to taper therefrom toward the periphery. The diabetic lesion, however, differs unequivocally from this type of progressive thickening in that it may involve a single lobule at the very periphery of the glomerulus and at a pole directly opposite that of the hilus (fig. 3). Furthermore, if such a lesion is followed serially, it generally will be found that the hyalin ends abruptly in the lobule involved and is not part of a continuous bar of hyalin extending to the vascular pole. As a matter of fact, in some instances the hilus and much of the remainder of the glomerulus may be practically spared. It is this

12. Kimmelstiel, P.: Am. J. Path. **11**:483, 1935.

characteristically focal, irregular rather than diffuse thickening which serves as one of the chief differential points in sections stained even with hematoxylin and eosin.

2. The silver stain demonstrates the deep brown coloration or the generally regularly laminated fibers of the diabetic lesion in distinct contrast to the relatively scanty and dissimilarly arranged silver fibers in the capillary loops and connective tissue of the nonspecific sclerotic portions of the glomeruli. The silver fibers present in Bowman's capsule offer no diagnostic difficulty (fig. 4).

3. The selective resistance of the diabetic lesion to tryptic digestion is highly characteristic (fig. 5).

4. There is the supplementary finding of inconstantly but, in severe involvement, extensively thickened and narrowed efferent arterioles. These are stated to be normal in nondiabetic hypertensive kidneys.

In other words, these distinctive focal changes observed in the kidneys of about 33 per cent of diabetic patients over the age of 40 (Siegal and Allen [7]) are not simply the changes of advanced nephrosclerosis. And, to be sure, one does not find them even in the most markedly contracted kidneys of the nondiabetic patient, where one would logically expect to find them if they were in fact a progressive stage of glomerular atrophy.

Glomerulonephritis.—Kimmelstiel and Wilson [1] mentioned that this "same histological picture [referring to the glomerular lesion observed in diabetic kidneys] frequently complicates glomerulonephritis." Accordingly, the kidneys in 34 unselected cases of subacute and chronic glomerulonephritis were studied. Of the 34 kidneys, 5 showed the so-called intracapillary type of glomerulonephritis, in which lesions that might be regarded as suggestive of the diabetic change were found. Kimmelstiel and Wilson [1] distinguished the nephritic from the diabetic lesions on the basis of the wrinkling, splitting and blurring of the capillary basement membranes in the former. In the diabetic glomerulus, "the basement membrane may be delicate like the normal one, or somewhat thickened, but is never wrinkled or split." [1] However, one is compelled to state that not infrequently split and wrinkled capillary basement membranes have been found in diabetic glomeruli, which are shown by serial sections to be part of incomplete or fully formed lesions. Moreover, it is felt that one can differentiate with confidence the glomerulonephritic lesions from those seen in diabetes with additional criteria. In the first place, in none of the nephritic kidneys were large lesions found; rather, they were uniformly small and arranged with a characteristic lobularity. When lesions are found to any extent in diabetic kidneys, they generally show much variability in size. In the second place, there is an appreciable difference in the type of argyrophilia of the lesions. The hyaline

bodies of the nephritic lesions are composed not of laminated silver fibers but of irregular strands and ringlets. Notwithstanding these differential features, there appears to be no reason to presume that the two processes are mutually exclusive, and it is to be expected that occasional cases of intracapillary glomerulonephritis will be found with superimposed diabetes in which the lesions of both are present.

Additional possible but easily eliminated sources of confusion are the so-called focal embolic glomerulonephritic lesions (Löhlein-Baehr) of subacute bacterial endocarditis and the degenerative, eosinophilic, often fatty changes seen in nephrosclerosis.

Intramural vs. Intercapillary Origin of the Diabetic Lesion.—The most difficult phase of this study is the determination of the morphogenesis of the lesion. Owing in good part to the observations of Zimmerman,[13] much attention has been focused on the intercapillary tissue which is said to lie between the basement membranes of the endothelial and epithelial cells. It is considered to be most abundant at the hilus, from which it fans out toward the periphery. It is apparently not regarded as a mantel of collagen surrounding the capillary wall but, rather, as analogous to a mesentery to which the capillaries are attached; hence, the name "mesangium." MacCallum [14] expressed the belief that the changes in glomerulonephritis and in arteriosclerosis and arteriolosclerosis of the kidney are due actually to a proliferation of the intercapillary connective tissue with compression and displacement of the capillaries. Kimmelstiel and Wilson [1] considered this to be the mechanism in the formation of the diabetic lesion, except that, as they stated, it did not involve fibroplastic proliferation but merely hyalinization and thickening of the preexisting collagen.

The observations recorded in the present study point toward an intramural origin of the hyaline thickening.[15] In the first place, one can make out endothelial basement membranes that are splitting or fraying into several layers which lead gradually or abruptly into a segment of hyalinized collagenous tissue. If such structures are followed serially, they may be seen to be portions of capillary walls which in succeeding planes become fused to form the diabetic lesion. This interpretation is made with awareness of Möllendorff's [16] impression that where the

13. Zimmerman, K. W.: Ztschr. f. mikr.-anat. Forsch. **32**:176, 1933.

14. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **55**:416, 1934.

15. It is of interest in this connection to recall the parenthetic observation on this lesion made by E. T. Bell and B. J. Clawson (Arch. Path. **5**:939, 1928). They regarded the process as a hyalinization not of the intercapillary tissue but of the endothelium.

16. von Möllendorff, W.: Ztschr. f. mikr. Anat. **11**:46, 1930; Der Bau des Nephrons, in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 7, pt. 1, pp. 40-43.

mesangial tissue apposes the capillary basement membrane the two may be indistinguishable. On the other hand, in order to indicate the real possibility for the misinterpretation of intramural as intercapillary tissue, one may recall that such observers as Möllendorff,[16] Borst [17] and Clara [18] concluded that some of the collagenous masses which Zimmerman regarded as lying between the capillaries were, in reality, tangential sections of capillary walls. Borst based his criticism on the demonstration in serial sections of these masses within capillary walls about obvious vascular lumens, as has been done in the present study.

In view of the possibility, as expressed by Möllendorff,[16] that the capillary basement membrane may be indistinguishable from the adjacent intercapillary fibers, it is considered not unlikely that the two may take part in the hyalinization. However, such intercapillary tissue is not to be regarded as an arborial, "axial" mesangium but rather as a mantle corresponding to an adventitia. This collagenous mantle is thickest at the hilus, gradually thins out toward the more peripheral portions of the capillaries, and is again formed in progressively increasing thickness as the efferent arteriole is approached. It appears likely that the reticular nature of this mantle accounts for its interrupted approximation to the capillary basement membrane. With this concept of the glomerular connective tissue, it becomes of little moment if it, too, especially near the vascular pole, takes part in the formation of the diabetic lesion, inasmuch as the genesis remains mural in effect.

These observations are quite in accord with McGregor's [19] view of the intracapillary origin of the hyalinized collagenous tissue in glomerulonephritis. McGregor believed, as a result of a careful, widely recognized study, that the hyaline fibers originated from the basement membrane of the capillaries. They may be so numerous as to obliterate the lumen of the capillary and so form the hyaline masses which are considered by Kimmelstiel and Wilson [1] to be intercapillary in location and to resemble strongly the diabetic lesion. It is felt from the current study that the mode of genesis of the two lesions is identical and quite along the lines suggested by McGregor. The only significant difference appears to be essentially quantitative, pertaining to the focal character and to the frequently greater length and characteristic arrangement of the fibers in the diabetic lesion.

Factors in the Albuminuria.—Albuminuria of a moderate to marked degree constitutes a major component of the clinical syndrome associated with widespread diabetic lesions of the kidneys. Is there any

17. Borst, J. G. G.: Ztschr. f. mikr.-anat. Forsch. **23**:455, 1931.
18. Clara, M.: Ztschr. f. mikr.-anat. Forsch. **40**:147, 1936.
19. McGregor, L.: Am. J. Path. **5**:559, 1929.

distinctive morphologic evidence in these kidneys which might furnish clues to the mechanism of the albuminuria? It is rather generally conceded that albuminuria is the result of abnormal capillary permeability. In cardiac decompensation, the slight albuminuria is attributed to passive congestion and anoxia. In thrombosis of the renal veins these factors are magnified, and the albuminuria may be marked. In glomerulonephritis, the mechanism is disputed, but recently Dunn [20] stressed the role of glomerular capillary dilatation and increased capillary pressure. In lipoid nephrosis, the morphologic basis for the albuminuria is obscure. In experimental animals, the albuminuria produced by the administration of epinephrine was considered the result of increased glomerular permeability due to anoxia following renal vasoconstriction.[21] In other words, the common denominator of these hypotheses appears to be anoxia and increased capillary pressure.

As previously stated, one observes in serial sections more or less dilatation of the peripheral capillary loops about most of the diabetic lesions. Occasionally, the loops are profoundly dilated and appear to be bursting with red blood cells (fig. 7). This has been observed, too, by Murakami.[2] In some, small fibrin clots and pools of laked blood are seen. Protein precipitate may lie within the lumen and close by in Bowman's space. The capillary basement membrane appears stretched to a fine line. This, then, represents the picture of stasis, and, it seems reasonable to add, probable anoxia and increased capillary pressure. The prime factor in this selective dilatation of the peripheral loops appears to be the stenosis produced by the thickened hyaline mass of the associated diabetic lesion which projects into the lumen of the capillary. It has been shown experimentally (Krogh [22]; Landis [23]) that stasis, anoxia and distention of capillaries in which there is an increase of pressure will bring about an increase of permeability, even to proteins. The narrowing of the efferent arterioles may be a contributory factor by virtue of the tendency toward increased intraglomerular pressure. Possible damage of the capillary wall as a consequence of its participation in the process of glomerulosclerosis may be still an additional factor. At any rate, in view of the frequency of these dilated loops and their association with the diabetic lesions, it seems not unreasonable to assign to them a significant role in the mechanism of albuminuria in these cases.

Efferent Arteriolosclerosis.—The efferent arterioles have been found to be definitely thickened and narrowed in kidneys with extensive diabetic lesions. The presence of efferent arteriolosclerosis is mentioned

20. Dunn, J. S.: J. Path. & Bact. **41**:169, 1940.

21. Starr, I., Jr.: J. Exper. Med. **43**:31, 1926.

22. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, Conn., Yale University Press, 1929, p. 335.

23. Landis, E. M.: Am. J. Physiol. **83**:528, 1928.

in the protocols of 2 cases of Newburger and Peters [5] and is indicated in the legend of a photomicrograph of 1 case of Anson's.[3] On the other hand, McGregor,[24] after study of serial sections of "the hypertensive contracted glomerulus," concluded that the efferent arterioles were normal in these ordinary nephrosclerotic kidneys notwithstanding the presence of afferent arteriolosclerosis.[25] In chronic glomerulonephritis there also appears to be a striking contrast between the "capillary-like" efferent arterioles and the thickened afferent arterioles (Oliver and Lund).[26] Therefore, the occurrence of appreciable efferent arteriolosclerosis in diabetic kidneys becomes a remarkable situation if McGregor's observation is found to hold universally. The important role in glomerular dynamics that is being attributed to the efferent arteriole (Smith and co-workers) [27] indicates that studies of filtration fractions, filtration rates and renal blood flow in these cases might be illuminating. Furthermore, in view of the presence of hypertension so commonly in the cases in which the diabetic renal lesions are widespread, this finding assumes an added interest, especially since it has been suggested that the pressor substance in experimental hypertension acts through constriction of the efferent vessels (Merrill, Williams and Harrison).[28] The interpretation of the significance of the narrowed efferent arterioles in this regard must be tempered with the finding of Corcoran and Page [29] in their studies of animals infused with renin that, although an increase in arterial pressure is accompanied by apparent constriction of the efferent arterioles, the degree of constriction does not parallel the rise in arterial pressure. Evaluation of the changes in the juxtaglomerular apparatus which have been noted in experimental animals (Goormaghtigh)[30] has been delayed until a systematic micrometric study can be done. Finally, no definite correlation was noted between the degree of efferent arteriolosclerosis and that of tubular atrophy.

Concerning the genetic relation of efferent arteriolosclerosis to the diabetic lesion it may be stated that this lesion is sometimes found in glomeruli with normal-appearing efferent arterioles, and, conversely, it is sometimes absent in glomeruli with thickened efferent vessels. In other words, the two lesions appear to be independent of each other.

24. McGregor, L.: Am. J. Path. **6**:347, 1930.

25. D. Loomis (Arch. Path. **22**:453, 1936), however, observed thickening of some of the efferent arterioles in her plastic studies of hypertensive kidneys, but she did not mention the presence or absence of diabetes.

26. Oliver, J. R., and Lund, E. M.: Arch. Path. **15**:755, 1933.

27. Smith, H. W.; Chasis, H.; Goldring, W., and Ranges, H. A.: J. Clin. Investigation **19**:751, 1940.

28. Merrill, A.; Williams, R. H., and Harrison, T. R.: J. M. Sc. **196**: 240, 1938.

29. Corcoran, A. C., and Page, I. H.: Am. J. Physiol. **126**:354, 1939.

30. Goormaghtigh, N.: Am. J. Path. **16**:409, 1940.

Correlation with Changes in the Pancreas.—The extent of the diabetic lesions was correlated with the degree of hyalinization of the islets of Langerhans. It was found that islet hyalinization occurred just as frequently and to as great a degree in the pancreases of diabetic patients without the glomerular lesions as in those with them. Incidentally, the hyalin of the pancreatic islets reacts unlike the hyalin of the diabetic glomerular lesion with the silver stain, but rather more like that of the nonspecific nephrosclerotic portions of the glomeruli. It was found, further, that in the cases in which the glomerular lesions were most extensive there was commonly marked arteriolosclerosis of the pancreas. On the other hand, in several such cases there was minimal involvement of the pancreatic arterioles. Moreover, there were numerous cases in which the diabetic glomerular lesions were not present but in which there was striking pancreatic arteriolosclerosis.

Occurrence of Diabetic Lesions in Nondiabetic Patients.—Hitherto, the lesion has been reported exclusively in patients with diabetes except for the single patient whose case was reported by Kimmelstiel and Wilson.[1] That patient died before an adequate study could be made. However, the present study includes 3 cases in which the lesion was found though glomerulonephritis was excluded and the diagnosis of diabetes could not be made clinically. One of these cases was among the series of 100 consecutive cases of hypertension; the other 2 cases were observed by chance. All 3 patients had a history or an actual record of hypertension. One patient had, in addition to hypertension, marked albuminuria and azotemia. The second patient died several hours after admission with a blood sugar determination of 260 mg. per hundred cubic centimeters. However, there is the disconcerting fact that this patient received 50 cc. of a 50 per cent solution of dextrose intravenously. Although it is highly unlikely that the withdrawal of the sample of blood for the determination of sugar followed the administration of dextrose, nevertheless the remote possibility that this did occur makes the diagnosis equivocal. Observations made after an admission three months previously revealed no evidence of diabetes in this patient. The third patient died immediately after admission without any laboratory work-up. The tissues in these cases were not fixed in absolute alcohol, so that one cannot adequately determine the distribution of glycogen. Incidentally, the pancreatic islets were moderately to markedly hyalinized and the efferent arterioles thickened in 2 of these instances. In other words, the glomerular lesions have been found in 3 cases in which the diagnosis of diabetes could not be made, nor, for that matter, excluded with certainty. It is pertinent in this connection to quote the statement of Newburger and Peters [5] "that it is not unlikely

that cases will be observed in which the diabetes is latent or even in which the sugar tolerance is not decreased."

Histologic Diagnosis of Diabetes.—Currently, the morphologic diagnosis of diabetes rests on an evaluation of the following criteria:

1. Hyalinization of the islets of Langerhans.
2. The presence of glycogen in the renal tubules and the liver cells.
3. The sparsity of glycogen in striated muscle.

The evaluation of these criteria is obviously difficult even under ideal conditions. Judgment is made more difficult, however, by (1) the occurrence of islet hyalinization in elderly nondiabetic persons (Wright [31]); (2) the frequent absence of glycogen from the kidney, due in part to the rapid postmortem disappearance of glycogen from tissues (one half to three quarters of the total amount within one hour, according to Yater, Osterberg and Hefke [32]; (3) the requirement of a special fixative for the glycogen stain, and (4) the not infrequent absence of these features in undoubted cases of diabetes. Consequently, the diabetic glomerular lesion acquires additional significance as a morphologic means of diagnosis of diabetes. Contrary to Kimmelstiel and Wilson,[1] who stated that this lesion occurred infrequently, it has been found in the present study in fully 33 per cent of 105 cases. This high frequency coupled with the ready recognizability of the lesion, makes it a relatively reliable criterion, which is still further enhanced if supplemented with the criteria previously used. There is the additional advantage that the lesion occurs predominantly in patients whose diabetes is mild and may perhaps be missed clinically.

Hyalinization.—As a final word, one feels obliged to remark that the observations herein recorded may perhaps seem unnecessarily detailed. However, the lesion acquires importance not only because it is related to the clinical syndrome of diabetes but because its interpretation is an integral part of the general problem of hyalinization. Efforts directed at a more definitive use of the currently nebulous, generic term "hyalin" may serve to clarify certain obscure histologic processes as well as terminology. For example, it is obvious that different types of hyalin may look similar when stained merely with hematoxylin and eosin. Nevertheless, further studies may reveal that one form of hyalin is predominantly collagenous, whereas the other is fatty, digestible with trypsin and azocarminophilic. These differences may reflect possibly different stages of the same process; or perhaps they may indicate that the provocative agent acts selectively on one site as compared with

31. Wright, A. W.: Am. J. Path. **3**:461, 1927

32. Yater, W. M.; Osterberg, A. E., and Hefke, H. W.: Proc. Staff Meet., Mayo Clin. **13**:243, 1928.

another because of certain local, conceivably hemodynamic conditions. Undoubtedly, when future investigators relieve the stalemate of inadequate technic, particularly in the field of organic and physical chemistry of tissues, morphologic niceties will acquire additional significance.

SUMMARY

The kidneys from 105 consecutive patients with diabetes, 100 consecutive nondiabetic patients with hypertension, 100 consecutive nondiabetic, nonhypertensive patients and 34 patients with glomerulonephritis were studied with a variety of special stains, principally from the point of view of glomerular changes.

A characteristic focal glomerular hyalinization of the type described by Kimmelstiel and Wilson was found in the kidneys from 33 per cent of diabetic patients over the age of 40. In addition, the lesions were present in 3 cases in which the diagnosis of diabetes could not be made or excluded with certainty. In 2 of these cases, the laboratory work-up was either inadequate or not done.

The diabetic lesion is easily distinguishable from the glomerulosclerosis of nephrosclerotic kidneys from nondiabetic persons and from the glomerular hyalinization of glomerulonephritic kidneys.

The diabetic lesion is regarded as a focal intramural glomerulosclerosis (i. e. arising *within* the capillary walls) rather than *inter*-capillary as it has hitherto been considered.

The finding of appreciable efferent arteriolosclerosis in the diabetic kidneys is recorded and its significance commented on, especially in view of its reported absence from kidneys of nondiabetic patients with hypertension.

It is suggested that the dilated, often intensely congested capillary loops constantly associated with the diabetic lesions and showing evidence of stasis are a significant factor in the genesis of the albuminuria which is characteristically present.

Detailed studies of the glomerular lesions are presented for the supplementary purpose of assigning definitive characteristics to the generic term "hyalin."

Because of the high incidence and clearcut character of the diabetic lesion, this lesion is offered as the most reliable criterion available for the histologic diagnosis of diabetes mellitus in cases in the age group over 40.

CONGENITAL ANOMALY OF THE CEREBELLAR VERMIS

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Developmental anomalies of the vermis of the cerebellum are rare if one may judge from the literature. A case which came to my attention is herewith reported because the patient presented the signs and symptoms of a tumor of the posterior fossa.

REVIEW OF THE LITERATURE

Anomalies of the vermis of the cerebellum range from incomplete development to absence of the structure itself. Failure of development has been reported by a number of observers. Rossi [1] described 2 cases; one of his patients was a woman aged 31 and the other was a boy 2 days old. A third case was reported by Fusari.[2] His patient was a woman of 48 with absence of the vermis and a greatly enlarged fourth ventricle, covered by a vascular membrane. According to Vogt and Astwazaturow,[3] who made a comprehensive review of the subject of congenital cerebellar deformities, these three reports were the only ones dealing with absence of the vermis up to the year 1912.

Obersteiner [4] in 1916 described an anomaly of the vermis in a 28 year old man who presented no evidence of cerebellar dysfunction. The two lateral lobes were continuous in the midline; the uvula and the nodulus were identified, but the lingula and the anterior medullary velum were absent. Obersteiner reported that the flocculi were probably absent in his patient, whom he regarded as presenting developmental failure of the paleocerebellum.

In 1931 Lyssenkow [5] published observations on a patient whose condition he designated as paleocerebellar aplasia. This patient, 25 years old, had never been able to walk, presumably as a result of his incoordination. The two cerebellar hemispheres were much smaller than normal. Although the flocculi were present, there was complete absence of the

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1. Rossi, M.: Sperimentale **45**:518, 1891; **46**:310, 1892.
2. Fusari, R.: Mem. r. Accad. d. sc. d. Inst. di Bologna **2**:643, 1891-1892.
3. Vogt, H., and Astwazaturow, M.: Arch. f. Psychiat. **49**:75, 1912.
4. Obersteiner, H.: Arb. a. d. neurol. Inst. a. d. Wien. Univ. **21**:124, 1914.
5. Lyssenkow, N. K.: Virchows Arch. f. path. Anat. **280**:611, 1931.

vermis. All of the cerebellar nuclei were present, but the dentate nuclei were incompletely developed. The peduncles of the cerebellum, particularly the restiform bodies, were reduced in size. The inferior olives, the arcuate and the red nuclei showed atrophic changes.

Pines and Surabaschwili [6] in 1932 reported an example of partial agenesis of the vermis which resembled the case I shall describe. Their patient was a 24 year old mentally deficient man whose speech was inarticulate and who exhibited a great deal of motor activity. They noted that the vermis was incompletely developed; it consisted only of the lingula, lobus centralis and culmen and the anterior portion of the declive and the nodulus. The greatly widened fourth ventricle was covered by a membrane (pia mater), which stretched from one cerebellar hemisphere to the other. The right dentate nucleus and the left inferior olive were abnormally small. They concluded that there had probably been no connections between the pontocerebellar tracts and the vermis, least of all the inferior vermis; that not all the fibers of the brachium conjunctivum arose from the dentate nuclei; that the existence of connections between the spinocerebellar tracts and the inferior vermis was very doubtful; finally, that there were no connections between the inferior olive and the vermis.

In 1933 Castrillón [7] described, under the title of paleocerebellar aplasia, another case resembling mine: His patient was a 59 year old mentally deficient woman who was subject to fainting spells and severe headaches and showed Romberg's sign. At autopsy incomplete development of the vermis was observed together with an enlarged fourth ventricle, which, like that of Pines and Surabaschwili's patient, was covered by a membrane which extended from one cerebellar hemisphere to the other. Castrillón reported that the flocculus was absent, although his illustrations indicate that this structure was present. The portions of the vermis which were identified were the anterior lobe of the vermis, the central lobe, the culmen and the declive. The remainder of the vermis tapered off into a rudimentary structure which probably contained the lobus medialis. He described partial atrophy of the dentate nuclei (especially the right), absence of the roof nuclei and absence of the arcuate fibers. He added that the motor region of the cortex and the pyramidal tracts were hypertrophied in order to compensate for the lack of cerebellar development.

The case reported by Scarff [8] in 1933 might be included in this group of cerebellar anomalies, although there was no pathologic examination. That such conditions also occur in animals was evidenced by the report of

6. Pines, L., and Surabaschwili, A.: Arch. f. Psychiat. **96**:718, 1932.
7. Castrillón, H. A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **144**:113, 1933.
8. Scarff, J. E.: J. Nerv. & Ment. Dis. **78**:400, 1933.

Bertrand, Medynski and Salles,[9] who described complete absence of the vermis in a dog and noted that Lesbre and Forgeot [10] reported a defective vermis in a calf. Dow [11] recently described partial agenesis of the vermis in 2 dogs.

REPORT OF A CASE

R. M., a 16 year old boy, was admitted to the University Hospital complaining of dizziness and severe pain in the back of the head and neck. He was the only child in the family. His parents were in good health, and there was no family history of a similar disorder. His birth had been normal. He sat at the age of 5 months and began to talk at the age of 10 months. He learned to walk at the age of 14 months and appeared to have no difficulty in locomotion. When the patient was 12 months of age, his father picked him up and suddenly lowered his head, whereupon the patient had a generalized convulsive seizure. At the age of 6 years he contracted measles and bronchopneumonia, from which he apparently made a good recovery. Shortly afterward, however, he began to have attacks in which he would suddenly throw his head to the left and would turn around once or twice. He seemed to have "blank" and "obstinate" spells of short duration. He experienced dizziness after strenuous activity or whenever he lay on his back. He was able to ride a bicycle in a normal manner. He received average grades in school. He was admitted to the University Hospital at the age of 12 years with a skin eruption diagnosed as seborrheal dermatitis and remained in the hospital fifteen days; during this time no abnormalities of locomotion or behavior were noticed.

He was again admitted to the hospital in 1933, at which time he related how two weeks previously he had engaged in a neck-strengthening contest with another boy. The day following these exercises he experienced intense dizziness and a few days later had headache and pain in the back of his neck. On admission to a local hospital the only observations of any consequence were that he had a stiff neck and a pulse rate of 48 per minute. A lumbar puncture was reported to have shown nothing abnormal. He improved somewhat, but within a few days he again complained of severe pain in the back of the neck and dizziness. The pain became steadily worse.

On admission he was moaning and groaning because of pain in the back of the neck. The temperature was 99 F., and the pulse and respiratory rates were 88 and 22 per minute, respectively. Because a mydriatic had previously been instilled, his pupils were widely dilated and did not react to light. The ocular rotations were normal, and there was no evidence of nystagmus. The fundi showed no evidence of papilledema. The other cranial nerves were normal. The neck was held stiffly, and its rotations were painful and limited, particularly in anteflexion. The chest and abdomen were normal. The blood pressure was 120 systolic and 75 diastolic. The strength of the extremities was normal, and there was no asynergy of the extremities. The deep reflexes were normal, and the response to plantar stimulation was flexion. Cutaneous sensation was normal throughout. His gait, however, was ataxic, and he swayed noticeably in the Romberg test.

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9. Bertrand, I.; Medynski, C., and Salles, P.: Rev. neurol. **66**:716, 1936.

10. Lesbre, F., and Forgeot, E.: Rev. gén. de méd. vét. **6**:198, 1905; cited by Bertrand, Medynski, and Salles.[9]

11. Dow, R. S.: J. Comp. Neurol. **72**:569-586, 1940.

The urine had a specific gravity of 1.022, was acid in reaction and contained no albumin, sugar or blood. The erythrocyte count was 5,120,000; the leukocyte count, 16,100; the hemoglobin value was 100 per cent (Sahli). The blood smear revealed no abnormalities. The Wassermann and Kahn reactions of the blood were negative. The initial pressure of the spinal fluid was 105 mm. of water with the patient in the horizontal position, and a prompt rise and fall occurred with compression and release of the jugular vein; the fluid contained a trace of globulin (Pandy test) but no cells or blood; it gave a negative Wassermann reaction. Roentgenograms of the cervical spine and the base of the skull showed nothing abnormal.

The patient continued to complain of severe pain in the neck and back of the head. On the day following admission, he suddenly collapsed, his respirations ceased, his pulse became very slow and poor in quality, and he became intensely cyanotic. He resumed breathing after several minutes of artificial respiration. The next day he was observed to have early choking of the optic disks and a coarse horizontal nystagmus when he looked to the right. Three days later he had another attack, during which he was cyanotic for twenty-five minutes. A diagnosis of neoplasm of the posterior fossa was made. He was then transferred to the department of neurosurgery and was operated on by Dr. Hyndman.

The posterior fossa was exposed. On incision of the cerebellar dura about 5 ounces (.1480 L.) of cerebrospinal fluid gushed out. On opening the cerebellar dura more widely, the surgeon came on a large cavity, in the depths of which he identified the cerebellar hemispheres, which were widely separated. The vermis was apparently absent, and the roof of the fourth ventricle and the cerebellar cistern were combined to form one large cavity. Realizing that the abnormality was a congenital malformation, the surgeon decided that no further surgical procedures were indicated, and accordingly closed the wound.

After the operation the patient experienced so much difficulty in swallowing that it was necessary to feed him through a tube. His respirations were rapid and labored, and he grew steadily worse. Five days after the operation he died of pneumonia and bulbar paralysis.

The autopsy five hours after death revealed lobular pneumonia with scattered abscesses in the lungs. No congenital anomalies were present except in the posterior fossa. The gyri were flattened and the sulci narrowed. The occipital lobes were displaced in an outward and upward direction by pressure from below. The splenium of the corpus callosum showed displacement forward and upward. The tentorium was present.

The infratentorial structures were of singular interest (fig. 1). The fourth ventricle was greatly dilated because of the absence of a portion of the vermis which normally covers it. As a result of this defect the lateral lobes of the cerebellum had the appearance of being rotated outward. The anterior aspect of the roof of the fourth ventricle was covered by vermis which projected backward approximately one third of the usual distance. The rest of the fourth ventricle was covered by a thin membrane, which was attached to the vermis anteriorly, extended outward to the lateral aspect of each cerebellar hemisphere and then reached posteriorly and inferiorly as far as the medulla. The lateral recesses of the fourth ventricle were patent. The posterior and inferior portions of the sac had been removed at operation.

The cerebellar hemispheres were somewhat smaller than usual, but they retained their normal configuration. The maximum measurements of each cerebellar hemi-

sphere were: vertical, 4 cm.; lateral, 4 cm.; anteroposterior, 5 cm. The well developed cerebellar tonsils projected conspicuously into the cavity of the fourth ventricle. Each flocculus was readily identified in its normal position, immediately posterior and lateral to the lateral recess of the fourth ventricle. The peduncle of the flocculus on each side could be traced upward and forward in front of the tonsil and continued in attenuated form until it reached the roof of the fourth ventricle, where it joined the thin tongue of cerebellar tissue, which formed the most posterior aspect of the vermis.

Coronal sections of the anterior halves of the cerebral hemispheres revealed a moderate grade of internal hydrocephalus involving the lateral and third ventricles.

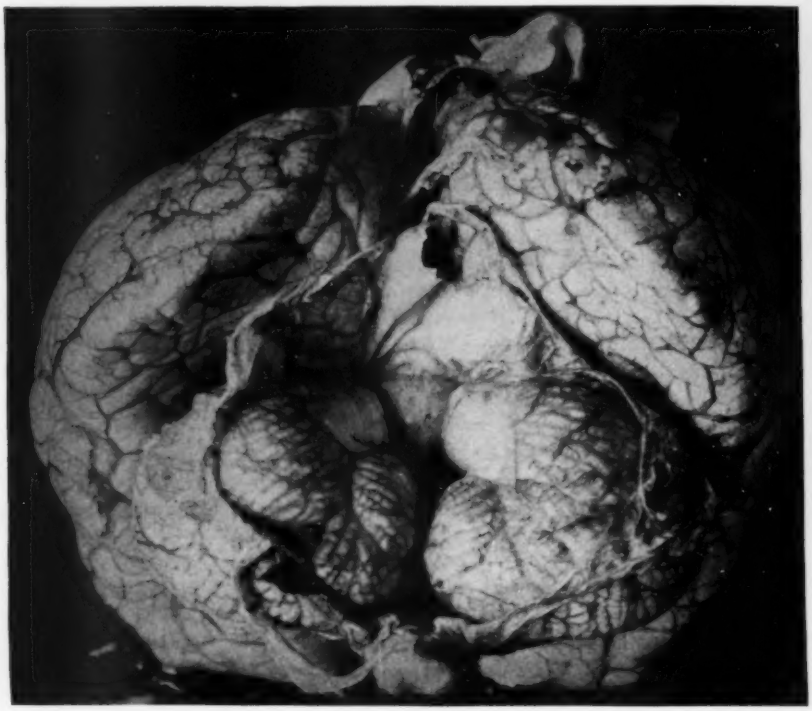

Fig. 1.—Posterior view of the brain, showing absence of the posterior portion of the vermis, the greatly enlarged fourth ventricle and the membrane extending from the vermis posteriorly and laterally over the cerebellar lobes and inferiorly to the medulla.

Midsagittal section of the remainder of the brain (fig. 2) showed that the rudimentary vermis was crowded forward into the space between the quadrigeminal plate and the splenium of the corpus callosum. The vermis measured 3 cm. in its anteroposterior and 3.5 cm. in its vertical diameter. The incisure of the inferior surface of the vermis (incisura fastigii) opened in a posteroinferior direction. The primary fissure could be identified but not as easily as in control specimens. The lingula, central lobule and culmen were present, although they were compressed to a rather marked degree and were considerably smaller than normal.

The divisions of the posterior lobe were less definitely distinguished, but I felt that I could identify a rudimentary median lobe as well as a poorly developed pyramis, uvula and nodulus. The nodulus was represented by a thin tongue of cerebellar tissue which extended into, and became continuous with, the membrane forming the roof of the fourth ventricle. Identification of this structure as the nodulus was aided by the fact that it received the peduncle of the flocculus. No choroid plexus was present in the roof of the fourth ventricle, although small amounts of plexus were present in the lateral recess on each side.

Each half of the cerebellum and brain stem was sectioned coronally. Representative sections from the right side were stained with myelin sheath stain

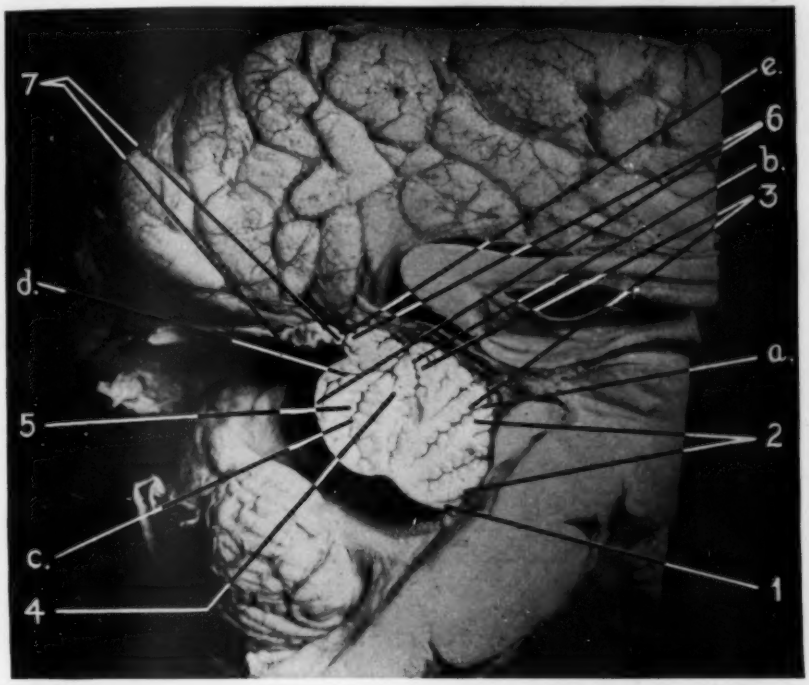

Fig. 2.—Midsaggital section of the posterior half of the brain, showing upward displacement of the splenium of the corpus callosum, the enlarged fourth ventricle and the anomalous vermis. *1*, lingula; *2*, lobulus centralis; *3*, culmen; *4*, median lobe of Ingvar; *5*, pyramis; *6*, uvula; *7*, nodulus; *a*, preculminate fissure; *b*, fissura prima; *c*, prepyramidal fissure; *d*, fissura secunda; *e*, uvulonodular fissure (medial) part of posterolateral fissure of Larsell).

(Weigert-Pal method). Those from the left side were stained with cresyl violet, hematoxylin and eosin and Masson's [12] trichrome stain.

Myelin sheath preparations showed that the medulla was normally developed in all respects. In particular, the dorsal and ventral spinocerebellar tracts, inferior

12. Masson, P.: J. Tech. Methods **12**:75, 1929.

olives, accessory olives, olivocerebellar fibers and internal arcuate and external arcuate fibers were entirely normal. The three cerebellar peduncles showed no abnormality.

Section through the cephalad portion of the inferior olive (fig. 3) disclosed the greatly enlarged fourth ventricle; the enlargement was the result of the absence of the vermis superiorly. The flocculus was well developed, and the dentate nucleus was present in its usual position. In a more cephalad direction at the level of the middle of the pons the vermis could be seen for the first time (fig. 4).

Fig. 3.—Cross section through the cephalad portion of the inferior olive. The flocculus and the dentate nucleus are well developed. The cavity of the fourth ventricle is very wide at this point. (Weigert-Pal; × 2.5).

The emboliform nucleus was well formed. No abnormalities were observed in the pons. In a slightly more cephalad location the globose nucleus and nucleus fastigii were identified. Section through the pontomesencephalic junction (fig. 5) showed normal brain stem structures; the anterior medullary velum, lingula and central lobule were readily identified. The vermis in particular was noticeably poor in myelin. Section through the mesencephalon at the level of the inferior colliculus showed moderate dilatation of the aqueduct.

Midsagittal section of the vermis showed that it was a small compressed structure, the divisions of which have been outlined in the gross description. The three layers of cerebellar gray matter were readily identified throughout the section. Patchy areas in which the Purkinje cells were sparse or entirely absent

Fig. 4.—Cross section through the pons at the level of the trigeminal nerve; the vermis here covers the fourth ventricle. (Weigert-Pal; × 3).

with narrowing of the other two layers were observed. The thin tongue of cerebellar tissue which formed a portion of the roof of the fourth ventricle became more and more attenuated until it merged with the membrane which I believe was the greatly

distended posterior medullary velum. This tongue of cerebellar tissue was identified as cerebellar gray matter with thin molecular and granular layers and an occasional Purkinje cell. The cytoarchitecture of the lateral lobe of the cerebellum was not unusual.

Sections through the medulla and cerebellum at the level of the lateral recess of the fourth ventricle showed that the membrane was very thin as it extended over

Fig. 5.—Cross section through the pontomesencephalic junction; the anterior vermis, particularly the lingula, is well visualized. (Weigert-Pal; × 2.5).

the medial and superior aspects of the cerebellar hemispheres. It consisted of a layer of glial tissue, external to which was a layer of connective tissue; the latter contained a moderate number of medium-sized blood vessels. The membrane was not intimately attached to the cerebellar cortex at any point but was joined only by a few septums of connective tissue, which became continuous with the pia-

arachnoid. A few flattened cells lined the inner aspect of the membrane. However, as the membrane dipped into the lateral recess of the fourth ventricle its medial aspect took on a continuous layer of flattened ependymal cells. In addition it was invaginated at that point by a small tuft of choroid plexus (fig. 6). The caudal limits of the membrane could be traced back as far as the junction of the medulla with the upper part of the cervical cord. There were no abnormalities

Fig. 6.—Cross section at the lateral recess of the fourth ventricle. The membrane is seen lining the medial aspect of the lateral lobe of the cerebellum and is invaginated by a small tuft of choroid plexus. (Masson's trichrome stain; × 8).

in the cellular structure of the medulla and pons. The vestibular nuclei and olives were normal.

COMMENT

Although this case resembled in gross appearance the ones reported by Pines and Surabaschwili [6] and Castrillón,[7] microscopic examination failed to show the changes in the dentate nuclei, inferior olives and

arcuate fibers reported by these authors. It is possible, furthermore, that sagittal views of the vermis in those cases would have revealed less actual defect in the vermis than was apparent grossly or in cross sections of the brain stem. It is my contention that in this case the major divisions of the vermis were present in rudimentary form. Hence, there seems to be no reason for regarding this as a case of paleocerebellar aplasia.

The failure of the vermis to close the fourth ventricle completely and the accumulation of cerebrospinal fluid in the fourth ventricle and over the superior aspect of both cerebellar hemispheres undoubtedly were responsible for the symptoms which preceded the fatal outcome. Certainly the volume of fluid became sufficient to compress and displace the occipital lobes, the vermis and the splenium of the corpus callosum and to exert enough pressure on the bulbar centers to cause the patient's collapse. The relationship between the acute phase of the patient's illness and the neck-strengthening contest is not entirely clear. It is evident, however, that the increase in venous and cerebrospinal fluid pressure which occurred during the period of exertion was an important factor in the development of his acute symptoms. Such exacerbations, which are known to occur in cases of tumor of the posterior fossa, are sometimes initiated by straining on the bedpan.

The patient's early difficulties, namely, his dizziness after exertion and when lying on his back, and the late manifestations of dizziness and ataxia would indicate that the midline cerebellar and vestibular structures were those principally involved in the production of his symptoms. It is reasonable to suppose that the symptoms were related directly to the mechanical effect of the accumulated fluid on the most vulnerable portions of the cerebellum and brain stem, namely, the nodulus, vestibular nuclei and, later, the respiratory center.

An embryologic explanation for the defect is possible. The failure of the flocculonodular lobe to fuse completely in the midline affected also the neighboring parts of the posterior lobe of the corpus cerebelli, namely, the uvula, pyramis and median lobe of Ingvar. The anterior lobe, which was phylogenetically and embryologically the first to develop, showed the least involvement. In addition the distention of the fourth ventricle with cerebrospinal fluid during embryonic development prevented the normal infolding of the nodulus, and thus maintained it as a thin structure. Dow [11] has concluded from study of animals that:

> In partial agenesis of the vermis . . . the formation of the cerebellum was possibly arrested at a slightly later stage after the cerebellar commissure had begun to form. This would allow a greater or less number of folia in the anterior lobe, beginning with the most anterior, to form across the midline in a fairly normal manner. . . . the posterior lobe of the corpus cerebelli is the last to be developed.

Failure in development at this stage would prevent the formation of the nodulus, the uvula, pyramis, declive and tuber vermis and the lobulus simplex. The parts which could go on to relatively normal development would be the hemispheres, the paraflocculi, the flocculi and the more anterior folia of the anterior lobe.

SUMMARY

A 16 year old boy who suffered from epileptic seizures and dizziness following exertion and changes in position was admitted to the hospital with acute symptoms which had developed after a neck-strengthening contest. At operation he was observed to have what was apparently a defect of the vermis with a greatly enlarged fourth ventricle. Autopsy revealed a moderate degree of internal hydrocephalus, generalized narrowing of the sulci and flattening of the gyri, and displacement and compression of the occipital lobes, of the splenium of the corpus callosum and of the vermis of the cerebellum. The vermis, however, was present in rudimentary form with all of its major divisions. The relationship between the patient's symptoms and the anatomic defect and a possible explanation for the abnormality on embryologic grounds are discussed.

ANATOMIC FINDINGS IN THE HEART IN COMBINED HYPERTENSION AND SYPHILIS

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The heart was studied in 33 cases of cardiac dysfunction associated with combined hypertension and syphilis without aortic regurgitation. The cardiac lesions observed in this series included a variety of changes, few of which were syphilitic. Incidentally it was noted that the clinical course in such cases is strikingly similar to that of syphilitic aortic regurgitation with cardiac failure.

The material studied was obtained in 33 cases in which autopsies were made at City Hospital, Welfare Island, New York, between 1928 and 1939. Five criteria were used in its selection: clinical evidence of cardiac failure, chronic hypertension, competency of the aortic valve, pathologic or serologic evidence of syphilis and in the kidneys histologically observed vascular changes compatible with hypertension. The blood pressure readings chosen were a systolic reading of not less than 160 mm. of mercury or a diastolic of 100 mm. of mercury or more. We considered the aortic valve competent when a normal or high diastolic pressure was present. The diagnosis of syphilis was made on the presence of syphilitic aortitis at necropsy, on not less than two positive Wassermann reactions of the blood serum or spinal fluid or on a combination of both anatomic and serologic observations.

Fifteen or more blocks were taken from the heart in 30 instances. They were chosen so as to include representative portions of the coronary arteries, the myocardium, all the valves and the conduction system. In 3 cases only five blocks were taken. In some instances many sections were cut from individual blocks. Hematoxylin-eosin was used as the routine stain. When indicated, Gram stains were done. The Levaditi method was carried out in 19 cases.

The incidence of combined syphilis and hypertension has received scant notice in the literature. White [1] and Fishberg [2] barely mentioned that it is observed. Among 436 cases of syphilis, Stone and Van Zant [3]

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1. White, P. D.: Heart Disease, New York, The Macmillan Company, 1935.
2. Fishberg, A. M.: Heart Failure, ed. 3, Philadelphia, Lea & Febiger, 1935.
3. Stone, C. T., and Van Zant, F. R.: J. A. M. A. **89**:1473, 1925.

found hypertension in 28.3 per cent; in none was there evidence of syphilitic heart disease. In McElroy's [4] series of 100 cases of essential hypertension, 5 per cent were syphilitic. In a survey of 666 hypertensive patients Horine and Weiss [5] found 21 per cent with positive serologic reactions. There was a greater preponderance among the Negro than among the white patients—30.4 per cent in contrast with 9.4 per cent. These authors also noted that at necropsy the condition of the heart was characteristic of hypertension and that syphilitic heart disease was absent. We have been unable to find any report dealing with histologic observations in this type of case.

CLINICAL DATA

The ages ranged from 27 to 71 years. The average age was 49 years. In 22 cases the ages fell between 35 and 55 years.

There were 22 men, 10 women and 1 pseudohermaphrodite whose internal genital organs were male. Of the men, 10 were white and 12 colored. Four of the women were white and 6 were colored. As the pseudohermaphrodite was a Negro, 19 of the 33 patients were Negroes.

A history of chancre was obtained in 11 cases. The onset of the cardiac failure occurred within five years after the chancre in 1, between five and ten years after in 2, between ten and twenty years after in 1, between twenty and thirty years after in 4 and more than thirty years after in 3.

The Wassermann reactions on blood serum or spinal fluid were positive in 21 cases and negative in 12.

The systolic blood pressure ranged from 160 to 270; the average was 195. The diastolic blood pressure ranged from 70 to 170, with an average of 120. In 30 instances it was 100 or over. In 3 cases the pressures were lower than 100—98, 96, 70; in all, the systolic pressures were between 170 and 200.

Thirty-one patients were admitted to the hospital in cardiac failure, usually of severe degree. The anginal syndrome and dyspnea were the most common manifestations. In 30 instances the chief complaints were "asthma" and nocturnal paroxysmal dyspnea. Precordial or substernal pain was present in 21; in 16 the pain was very severe. Edema of the extremities and enlargement of the liver were prominent in 13, three times associated with ascites. Cough, expectoration or other evidence of respiratory embarrassment was present in 12. Dizziness, headache, visual disturbances and other neurologic symptoms were prominent in 5 cases. In these 5 cases the patients proved to have uremia in addition to the cardiac failure.

Two of the patients were admitted to the hospital for neurologic conditions, one because of residual hemiplegia, the other for the tabetic form of dementia paralytica. Cardiac failure developed in these patients while they were under observation, and both presented the syndrome of acute left ventricular failure without anginal pain.

Electrocardiographic tracings were recorded in 24 cases. Left axis deviation was found in 19, right axis deviation in 1 and no deviation in 4. The last 5 were all cases of rheumatic heart disease. QRS abnormalities, usually slurring

4. McElroy, cited by Horine and Weiss.[5]

5. Horine, E. F., and Weiss, M. M.: Am. Heart J. **6**:121, 1930.

and notching, occurred in 11. T wave changes were noted in all cases, chronic changes in 21 and acute changes in the ST segment in 3. Chronic auricular fibrillation occurred four times, twice in the rheumatic hearts. Intraventricular block was found four times and nodal rhythm once. There was no example of complete heart block.

The course of the disease tended to be progressive and unrelenting. Twenty-two patients died in the first attack of failure, 9 in the second and 2 in the third. The average duration of life after the appearance of cardiac symptoms was thirteen months in the first group and eighteen months in the second.

The response to therapeutic measures was usually unsatisfactory. The administration of digitalis resulted in complete therapeutic failure in 25 cases. In 8 instances, 4 of which were cases of auricular fibrillation, the response was satisfactory. Improvement in 3 cases was attributed to the use of mercurial diuretics and not digitalis. Antisyphilitic therapy was used in few instances. Bismuth preparations apparently had neither beneficial nor harmful effects. Neoarsphenamine was employed in 1 case, and the condition appeared aggravated.

Death was caused by cardiac failure alone in 16 cases. The failure was of congestive type in 12 and sudden in 4. In 17 cases other conditions were found associated with cardiac failure, as accessory causes of death. In 5 of these cases

Classification of the Hearts by Weight

300 to 399 Gm.	400 to 499 Gm.	500 to 599 Gm.	600 to 699 Gm.	700 to 799 Gm.	800 to 899 Gm.	900 to 925 Gm.
2	1	8	8	7	3	2

there were complete renal decompensation and uremia; in 6, extensive extracardiac infections, and in an equal number, partial renal failure and infection.

Evidence of extracardiac infection, exclusive of terminal pneumonia, was found at necropsy in 25 cases. There were single foci in 10 cases and multiple foci in 15. In all, a total of 44 separate sites of infection were present. The genitourinary system accounted for 19 and the respiratory system for 12. Markedly carious teeth, decubital ulcers and in 1 instance peritonitis contributed to the remainder. It was of interest to note, however, that fever had been present in only 10 of the 25 cases in which infection was a feature, and in only 2 was it prominent. The patients in these 10 cases were all in the younger age groups.

PATHOLOGIC ANATOMY

Analysis of the pathologic observations in the 33 hearts disclosed a great variety of lesions, not only from the standpoint of the multiplicity of changes but from that of the combinations of lesions that were present in the hearts. Rare was the heart in which one process was the only operative factor. In the majority, the most important lesion could easily be determined. Almost every possible type of cardiovascular disease was represented.

Hypertrophy of the heart tended to be extreme. The weights ranged from 325 to 925 Gm., the majority being over 500 Gm. The data are charted in the accompanying table. The weights of 2 hearts were not recorded.

Judged on a histologic basis, hypertrophy of the fibers was marked in 25 and moderate but definite in 4 hearts. Left ventricular hypertrophy predominated in 25 and right ventricular in 3. Both ventricles were equally affected in 1 heart. In the last 4 hypertrophy occurred with rheumatic lesions. In 28 hearts there was a correspondence between weight and histologic appearance. The smallest heart, found in a woman weighing only 90 pounds (12.7 Kg.), had histologic evidence of hypertrophy. In 2 hearts microscopic examination failed to reveal evidence of hypertrophy although the weights were above normal. One of the hearts was very edematous, the water content probably explaining the discrepancy. The explanation of the discrepancy in the other was obscure.

Syphilitic involvement of the commissural region of the aortic valve was present in 13 hearts. Syphilitic aortitis was found in 30. Severe arteriosclerotic changes were combined with syphilis in 17. In 3 hearts the aorta was perfectly normal; neither gross nor microscopic involvement by syphilis was demonstrable. The 3 patients were women who had persistently positive serologic reactions.

Rheumatic valvulitis was found in 4 hearts. In 1, a stenotic mitral lesion was proved microscopically to be completely healed. Chronic active valvulitis of the aortic and mitral leaflets was present in the second. Histologic examination disclosed acute mitral valvulitis in the third and acute panvalvulitis in the fourth. In none of the hearts was there combined syphilitic and rheumatic disease of the same valve. Two hearts had acute endocarditis involving an aortic valve with commissural syphilis. An aortic valve stenosed by arteriosclerosis was found in 1 heart. All the valves were normal in 15.

Atresia or stenosis of the coronary mouths was caused by syphilitic aortitis in 3 cases, by arteriosclerotic plaques in 2 and by combined syphilis and arteriosclerosis in 3. Rigidity of the mouths from arteriosclerosis without change in the caliber was found twice, once with a recent occlusion. The mouths were normal in the remainder. In the majority of the cases, therefore, the coronary mouths were either normal or widened in diameter and were similar to those usually seen in the hypertensive heart.

Arteriosclerosis of the superficial coronary arteries was marked in 18 hearts, moderate in 8 and absent or slight in 7. Fresh occlusion in markedly sclerotic arteries was present in 2, one being the heart with occlusion of the coronary mouth. An old recanalized thrombus was found once. The adventitial reaction found in the sclerotic vessels tended to be intense and was more marked than that seen in the nonsyphilitic hypertensive heart, particularly in comparison with one of the same age period. The reaction was mainly lymphoid with a few plasma cells. In 1 instance the reaction was predominantly plasmacytic with

some lymphocytes and a few monocytes. The lesion was very similar to that found in syphilitic aortitis, but a search for spirochetes failed to reveal any. The heart with acute rheumatic panvalvulitis had an extraordinarily acute panarteritis affecting all the superficial arteries. The walls were edematous, and the intimal and subintimal zones were densely infiltrated by polymorphonuclear cells that frequently penetrated into or through the media. The adventitia contained dense herds of lymphoid and polymorphonuclear cells. Bacteria were not found. A similar vascular lesion was reported by Gross and co-workers [6] in rheumatic heart disease and interpreted as periarteritis nodosa.

The intrinsic coronary arteries in 18 hearts were normal. Moderate to severe atherosclerosis of the intrinsic arteries was present in 3 hearts, in 1 of which atheromatous occlusions were found in the involved arteries in some areas. Acute thromboses occurred in otherwise normal vessels seven times; three times the thromboses were bacterial in nature. Gummatous lesions of the intrinsic arteries were present in the heart in which the involvement of the superficial vessels resembled the lesion seen in syphilis of the aorta. The search for spirochetes was unsuccessful. In the heart with acute panarteritis of the superficial vessels, similar extensive involvement of the intrinsic arteries was present. The brain, kidneys and liver had similar vascular lesions with occlusions, although none were found thrombosed.

The myocardium in practically every heart was affected by extensive changes. These could be divided into three general groups: infectious, ischemic and fatty degenerative. They have been separated into the dominant process and the accessory processes.

Syphilis was the probable cause of the myocardial damage in 1 instance.[7] This case was the one mentioned with syphilitic involvement of both superficial and intrinsic arteries. Also present were acute endocarditis of the aortic valve and acute mural endocarditis. In addition, there were acute lobar pneumonia, chronic pyelonephritis and uremia. Whether the mural endocarditis was syphilitic, possibly secondary to the arteritis, or whether it was secondary to the valvular endocarditis, could not be determined definitely. Both spirochetal and Gram stains were negative. We were inclined, however, to attribute it to the acute infection rather than to syphilis.

Acute rheumatic myocarditis, characterized by innumerable Aschoff bodies, was present in 6 hearts, 4 of which were mentioned in connection with the description of valvular lesions. In 3 instances, the rheumatic involvement was discovered on histologic examination; in 2, the valves

6. Gross, L.; Kugel, M. A., and Epstein, E. Z.: Am. J. Path. **11**:253, 1935.

7. This case was reported elsewhere by J. R. Lisa (Ann. Int. Med. **12**:198, 1939, case 15).

were unaffected. In 1 case the myocarditis was associated with a massive acute infarction without coronary occlusion. Miliary infarctions were present in small numbers in 2 others, once with acute mural endocarditis.

Diffuse interstitial myocarditis was found the dominant lesion in 2 hearts. In the first, it was an acute affair, characterized by widespread distribution of polymorphonuclear cells and acute myocardial degeneration. An infectious basis was strongly suggested by the type of exudate, although bacterial stains were negative. The kidney showed acute glomerulitis with crescent formation superimposed on nephrosclerosis and acute necrotizing arteriolitis. The second heart had a similarly extensive infiltration by lymphocytes and plasma cells, associated with fine scarring and subendocardial miliary infarctions. Acute mural endocarditis and pericarditis were also present. There were extracardiac infections—chronic pyelonephritis and acute salpingitis—and acute glomerulitis. The possibility that the chronic lesion was syphilitic could not be proved definitely, since a search for spirochetes was unsuccessful, but the character of the exudate suggested it. The acute lesions were most probably associated with the extracardiac infections.

Acute miliary necroses were found as the principal change in 2 hearts. They were widely distributed, scarcely any portion of the heart being uninvolved. This lesion was essentially an acute focal degeneration of segments of individual fibers, which preserved a ghostly shadow-like outline and were infiltrated by monocytes or polymorphonuclear cells. The remaining portions of the affected fibers and the adjacent myocardium were normal in appearance. It was difficult to determine the relationship of this lesion to infection, although infection was present in both cases. The coronary arteries were normal; therefore the lesion could not have had a sclerotic ischemic basis. In the first instance there was acute glomerulitis; in the second, a large decubital ulcer associated with a septic temperature.

Massive acute infarction was the predominant lesion in 6 hearts. The left ventricle was affected in 3, the left ventricle and interventricular wall in 2 and the interventricular wall alone in 1. Recent coronary occlusions were present in 3, twice of the left anterior descending and once of the posterior descending artery. Acute thromboses of the intrinsic coronary arteries were found in 2 cases; in addition to the massive infarct, miliary infarctions were also seen. Infection as a prominent feature could be demonstrated in only 1 case: Acute lobar pneumonia was followed by cardiac failure, which necessitated hospitalization. During the course in the hospital, a pharyngeal pustule developed, and at autopsy there were found recent occlusion of the posterior descending artery, acute cardiac infarction of the left ventricle and base of the interventricular wall, a small myocardial abscess of the interventricular

wall, acute pericarditis and acute suppurative embolic nephritis. The coronary arteriosclerosis was moderate.

Acute miliary infarctions formed the dominant myocardial alteration in 8 hearts. This lesion has been described in previous communications.[8] Marked coronary arteriosclerosis was present in 7 of these hearts, 3 with normal coronary mouths, 4 with one or both mouths narrowed. In 1 heart, sclerosis was absent, but both coronary mouths were narrowed. Extensive extracardiac infections were present in 7 of the cases, associated in 1 with bacterial thrombi of intrinsic arteries.

Widespread fatty degeneration of the myocardium was present in 4 hearts, all with severe arteriosclerosis of the coronary arteries. One heart, with extreme atheroma of the intrinsic arteries, was described in a previous paragraph. It had lesions of various types—a few miliary infarcts, widespread lymphocytic reaction of endocardium and epicardium, acute polymorphonuclear infiltration of the regions of the atrioventricular and sinoauricular nodes and acute glomerulitis. Two hearts had extensive fibrosis.

Myocardial changes which did not fall into any of the groups previously described were found in 4 hearts. As a whole, these changes could be considered only minimal, although in all the cases death was due to congestive failure. One heart had widespread molecular degeneration of the ventricular myocardium, increased prominence of the lipochrome and extensive fine scarring. Marked fragmentation and moderate edema were the only findings in a second heart. In another, there were only marked arteriosclerosis and extensive gross and microscopic fibrosis. In the last heart there was only very early acute pericarditis; the coronary arteries and myocardium were normal. There was acute focal glomerulitis. The only possible site of origin for an infection to account for the pericarditis and glomerulitis was the mouth; the teeth were markedly carious and the mouth foul. Although hypertrophy was absent, the blood pressure was 210 systolic and 115 diastolic, cardiac failure was pronounced, signs of uremia were absent, and the patient lived only four days after admission.

COMMENT

The clinical impression created by observation of this group of patients was so similar to that of the syphilitic group with aortic regurgitation in failure that the parallel could not be missed. The predominantly left ventricular type of failure, the large number dying in the first attack, the lack of response to digitalis and the short course—this is a classic recital of the progress of disease in the syphilitic group with aortic regurgitation and failure. The fact that cases in which

8. Lisa, J. R., and McPeak, E.: Arch. Int. Med. **65**:919, 1940. Lisa.[7]

there was aortic valvular incompetence were eliminated by the insistence on a normal or elevated diastolic pressure made this even more striking. There was but little, however, in the clinical symptom complex to indicate which type or types of pathologic changes would be encountered in any particular instance.

In seeking factors for the rapid, often fulminant cardiac dysfunction, which was rather uniform, we noted several features as being of prime importance: the extraordinarily severe and widespread manifestations of myocardial disease regardless of its character, the multiplicity of lesions found in individual hearts and the extensive extracardiac infections.

One heart presented a type of syphilitic involvement that is seldom observed in acquired syphilis. The coronary arteries are proverbially unaffected. Even in extensive aortic syphilis with atresia of the coronary mouth and incompetency of the valve, the arteries are usually found normal. In the pathologic material in this laboratory, only 3 other instances of syphilitic myocarditis have been encountered since 1927, and in none was the arterial tree involved by a lesion of this character. The rheumatic myocarditis also was much more extensive than is usually seen in the age period encountered in this series. The degree of the degenerative and inflammatory lesions of the coronary arteries was definitely out of proportion to that which would be expected in this age group either with or without hypertension. It was particularly so in the hearts in which acute rheumatic myocarditis was found.

The variety of lesions found in the myocardium was pronounced. Miliary infarctions were found in greater or less numbers, but not as the dominant finding, in 9 hearts; twice with massive infarction, acute rheumatic myocarditis and miliary necrosis; once each with acute interstitial myocarditis, syphilitic myocarditis and fatty metamorphosis. Interstitial myocarditis, acute, chronic or mixed, as a secondary pathologic finding was present in 10 hearts, thrice with miliary infarction and massive infarction, twice with acute rheumatic myocarditis and once with syphilitic myocarditis. Miliary necrosis as a finding of secondary importance was present only in the case of acute interstitial myocarditis.

The condition of the coronary arteries was the only pathologic finding showing a recognizable correlation with the clinical features. Angina was common in this group, occurring in 21 cases. The chief cause for cardiac dysfunction could be ascribed to ischemia of the myocardium in 14 hearts, resulting in massive infarction in 6 and miliary infarctions in 8. As miliary infarctions were present in 9 other hearts and gross scarring in 5 more, there were 28 hearts in which the results of diminished myocardial blood supply were apparent either as a primary or as a contributory cause of failure and death.

The relationship of infection, cardiac and particularly extracardiac, to myocardial lesions and congestive failure, is more controversial than that of coronary disease. It is recognized, however, that any acute infection may precipitate or intensify cardiac failure. Weiss,[9] in discussing the relationship of infection to heart failure, pointed out the not infrequent occurrence of cardiac invalidism or death following influenza or other infections of the upper respiratory tract. Although this relationship between infection and cardiac failure is well recognized by the clinician, discrepancies are reported between the symptoms of failure and demonstrable myocardial damage. Schmorl[10] declared that only a small group of hearts show histologic changes in the myocardium to correspond with postinfluenzal cardiac dysfunction. Weiss grouped Fiedler's myocarditis, myocardial abscesses and the effects of infection of the upper respiratory tract on the myocardium among diseases of the heart which are uncommon and not well recognized when met. Even in cases of endocarditis it is believed that the myocardial lesions are too insignificant to explain myocardial failure. Clawson[11] concluded that "myocarditis seldom occurs in the bacterial forms" of endocarditis. Middleton and Burke,[12] in a study of subacute bacterial endocarditis, stated that "thruout the major course of the disease the myocardium generally escapes serious damage."

There are isolated reports in the literature of demonstrable myocardial injury occurring in cases of infection and sepsis and associated with clinical evidence of cardiac dysfunction. Magill's[13] cases were reported as instances of syphilitic myocarditis, although he failed to demonstrate the spirochete in the myocardium. In the cases reported by Jonas[14] and by Taussig and Oppenheimer[15] a nonspecific granulomatous lesion of the myocardium of undetermined cause was found. Major and Wahl[16] reported a case of paroxysmal ventricular tachycardia with death in cardiac failure in which there was acute focal myocarditis of the right auricle, left ventricle and bundle of His. Scott and Saphir[17] reviewed the literature on this subject to 1930. Saphir[18] reported 2 cases of acute myocarditis associated with meningococcic

9. Weiss, S.: M. Clin. North America **23**:1323, 1939.
10. Schmorl, cited by Weiss.[9]
11. Clawson, B. J.: Arch. Int. Med. **33**:157, 1924.
12. Middleton, W. S., and Burke, M.: Am. J. M. Sc. **198**:301, 1939.
13. Magill, T. P.: Bull. Johns Hopkins Hosp. **57**:22, 1935.
14. Jonas, A. F., Jr.: Bull. Johns Hopkins Hosp. **65**:45, 1939.
15. Taussig, H. B., and Oppenheimer, E. H.: Bull. Johns Hopkins Hosp. **59**:155, 1936.
16. Major, R. H., and Wahl, H. R.: J. A. M. A. **86**:1125, 1926.
17. Scott, R. W., and Saphir, O.: Am. Heart J. **5**:129, 1929.
18. Saphir, O.: Am. J. Path. **12**:677, 1936.

septicemia and demonstrated the meningococci in the myocardial lesions. Most of the authors who have reported individual cases believe that such findings are rare. Saphir called attention to the difficulty of making the diagnosis of myocarditis by gross inspection of the heart and suggested that more extensive histologic study would reveal a greater number of such instances.

Saphir [19] later carried out such a study on 35 cases of subacute bacterial endocarditis. In 26 hearts there were extensive inflammatory myocardial changes and in 14 of these there were, in addition, Aschoff bodies. He did not state the number of patients suffering from cardiac failure. Buchbinder and Saphir,[20] in a study of 40 cases of subacute bacterial endocarditis, attempted to correlate the incidence of myocardial findings with that of congestive failure. Myocardial damage was extensive in all the hearts and associated with congestive failure in 30. The most frequent abnormality found was minute myocardial infarction. We believe that this lesion, called "organizing infarction" and illustrated in Saphir's article by photograph 8 is similar to that described in previous communications from this laboratory as the very acute and early necrotic phase of miliary infarction. Roesler and Soloff [21] have described a similar lesion. Myocardial abscesses, focal necrosis, perivascular inflammatory reaction and fibrosis were common. In 10 cases of their series there was no evidence of congestive failure although extensive myocardial damage was present. This apparently deterred the authors from concluding that there was a definite correlation between the incidence of congestive failure and the presence of myocardial damage. In 6 of the 10 cases, however, the course was interrupted by embolic accidents. It is conceivable, therefore, that the accidents may have appeared before failure could become clinically evident.

Clawson [11] and Middleton and Burke [12] included in their reports myocardial changes similar to those reported by Saphir, although their conclusions were that no correlation existed between the clinical findings of cardiac failure and the myocardial changes. Clawson studied 102 cases histologically and found evidence of myocardial inflammation in 34, an incidence which seems too high to consider insignificant. In the series of 88 cases studied by Middleton and Burke there were various types of myocardial damage, exclusive of rheumatic or fibrotic lesions, in 48.7 per cent. This also appears much too high to be insignificant. Clawson admitted that the histologic examination was inadequate. The extent of the histologic examination carried out by Middleton and Burke was not stated.

19. Saphir, O.: Am. J. Path. **11**:143, 1935.
20. Buchbinder, W. C., and Saphir, O.: Arch. Int. Med. **64**:336, 1939.
21. Roesler, H., and Soloff, L. A.: Ann. Int. Med. **9**:477, 1935.

Although the present series includes a group somewhat different from those studied by other investigators, our findings as to the degree of myocardial involvement correspond closely to those of Saphir and co-workers. As they discovered in subacute bacterial endocarditis, the lesions in the heart were characterized by the multiplicity of different pathologic processes. Although Saphir did not draw a sharp distinction between many of the lesions he described, his descriptions correspond to what we have called miliary infarction, interstitial myocarditis, miliary necrosis and abscess. He noted in his cases an intracardiac infectious origin. We found in our cases infectious processes that were not necessarily associated with the endocarditis, including acute and chronic infections of the upper respiratory tract and of the dental areas, acute pneumonias, chronic bronchiolitis and bronchiectasis, acute and chronic genitourinary infections and some of less frequent occurrence.

The ubiquity both of extracardiac pyogenic infection and of myocardial findings in sudden deaths has already been stressed in previous communications from this laboratory.[22] Helwig and Wilhelmy [23] reported 3 cases similar in nature.

The frequent occurrence of acute glomerulitis, which is recognized as a sequel of infection, also can be cited as evidence for the role of infection in the production of myocardial damage in this series. Myocardial abscess in the presence of an acute infarction also is very suggestive of the effect of active infection precipitating a vascular accident. In most of our cases coronary sclerosis was present. It is our belief that the sclerosis tended to render the heart more susceptible to the deleterious effects of infection, whether the infection was specific, i. e., rheumatic or syphilitic, or whether it was pyogenic, cardiac or extracardiac.

The present series and the cases reviewed from the literature have extensive myocardial damage as a feature in common. In most of the cases it was associated with congestive failure or sudden death due to cardiac disease. That a certain number of hearts should have extensive myocardial damage unassociated with clinical symptoms of cardiac disease is not surprising. One is too frequently impressed at necropsy with the extraordinary amount of myocardial damage unassociated with clinical findings regarded as indicative of cardiac dysfunction. We recognize that the heart has extraordinary ability to function in spite of severe damage and thus may present only minor signs, which are easily overlooked or undervalued.

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22. Lisa, J. R.: Urol. & Cutan. Rev. **43**:742, 1939. Lisa, J. R., and Hart, J. F.: Arch. Int. Med. **64**:43, 1939; New York State J. Med. **40**:705, 1940. Lisa.[7] Lisa and McPeak.[8]

23. Helwig, F. C., and Wilhelmy, E. W.: Ann. Int. Med. **13**:107, 1939.

SUMMARY

The anatomic findings of the heart in 33 cases of cardiac failure occurring in association with combined chronic hypertension and syphilis without aortic regurgitation are presented. The hypertensive patients with syphilis after cardiac failure supervened presented a clinical picture and course strikingly similar to that of patients with syphilitic aortic regurgitation and failure. The first type of patient responded poorly to therapy and had a bad prognosis. The conditions described occurred more frequently among colored than among white persons and more frequently among males than among females. The age range of the majority of the patients was between 35 and 55 years. Death was due to cardiac failure alone in half the cases and to cardiac failure and added factors of partial or complete renal failure or infection in the remainder. The arteriosclerosis of the coronary arteries was much more severe than that found in members of the same age group who had hypertension not complicated by syphilis. Hypertrophy tended to be extreme. The damage to the myocardial fibers was severe and extensive and included diverse pathologic processes. Syphilitic myocarditis was found in 1 instance. Extensive rheumatic myocarditis was present in 6 cases, twice without valvular involvement. Other lesions included massive infarction with or without coronary occlusion, acute and chronic interstitial myocarditis, acute endocarditis, acute miliary infarctions, acute myocardial necroses and acute abscesses. The role of infection, valvular or extracardiac, in the production of myocardial damage appeared of special importance. The presence of severe coronary sclerosis associated with chronic hypertension and constitutional syphilis appeared to render the myocardial fibers more susceptible to injury, such as that produced by a specific infection, as syphilis or rheumatic fever or a nonspecific pyogenic infection. The clinical syndrome of cardiac failure was associated with extensive demonstrable injury to the myocardium in almost all cases. The demonstration of myocardial damage depends on adequate histologic examination.

EFFECT OF PROLONGED ADMINISTRATION OF LARGE QUANTITIES OF SODIUM BICARBONATE ON THE KIDNEY OF THE DOG

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The alkalosis which occasionally complicates alkali therapy is almost always accompanied by an impairment of renal function. The nature of the renal disturbance and the mechanism of its production are not known, although various authors, including Stieglitz,[1] have expressed the opinion that it is related directly to anatomic changes in the kidney resulting from the continued use of alkali.

Various investigators have attempted to clarify this problem by studying the effect of alkali on the kidney of the experimental animal. Fischer [2] reported albumin, casts and red blood cells in the urine of rabbits given large amounts of tenth-normal sodium hydroxide and twice-molar sodium chloride. No histologic studies of the kidney are mentioned. Similar results were obtained with acids, urea, pyridine and certain amines. Nuzum and co-workers [3] kept rabbits on a diet of ground soybeans (protein content, 36 per cent) for two years. The nonprotein and urea nitrogen of the blood remained normal, and there was no evidence of renal damage. The authors' claim that persistent mild alkalosis injures the kidney is not supported by the data presented. Addis, MacKay and MacKay [4] fed 24 albino rats a diet containing 4 per cent sodium bicarbonate (in man, equivalent to 2,560 cc. of tenth-normal sodium hydroxide for almost one year. Twenty-four of the 72 urine specimens contained large amounts of blood. In 7 rats hydronephrosis developed in one or both kidneys, but in none was the condition so far advanced as to have produced appreciable renal atrophy. The blood urea nitrogen was normal, and there were no apparent microscopic differences between the kidneys of the control, acid and alkaline rats. Kellert [5] injected 12 cc. amounts of a 5 per cent sodium bicarbonate

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From the Department of Medicine, University of Chicago.

1. Stieglitz, E. J.: Arch. Int. Med. **41**:10, 1928.

2. Fischer, M. H.: Edema and Nephritis, New York, John Wiley & Sons, Inc., 1915, p. 428.

3. Nuzum, F. R.; Seegal, B.; Garland, R., and Osborne, M.: Arch. Int. Med. **37**:733, 1926.

4. Addis, J.; MacKay, E. M., and MacKay, L. L.: J. Biol. Chem. **71**:157, 1926.

5. Kellert, E.: Am. J. M. Sc. **167**:114, 1924.

solution intravenously in normal rabbits and in rabbits in which renal damage had been induced previously with 0.025 Gm. of potassium chromate, 10 cc. of 1:1,000 mercurous chloride and 0.002 Gm. of uranium nitrate. No anatomic evidence was obtained to indicate that sodium bicarbonate in the quantities used was injurious to the kidney. Suzuki [6] fed rabbits a diet of soybean husks and also injected 5 per cent pure sodium bicarbonate in distilled water intravenously and intraperitoneally. No unusual pathologic changes were noted. The kidneys were hyperemic; on microscopic examination, the epithelial cells were slightly turbid, and there were infrequent hemorrhages in Bowman's capsule.

The present investigation was undertaken to determine more conclusively than has been done previously the effect of alkalis on the structure of the kidney. For this purpose, large amounts of sodium bicarbonate were given orally and intravenously to a group of 9 dogs for periods ranging from thirty to two hundred and sixty-one days. The study is of particular interest in that a control histologic examination of one kidney of each animal was made prior to the administration of alkali.

METHOD OF STUDY

Thirteen healthy dogs of various body weights were selected for the experiments. One kidney was removed from each animal for control microscopic study. Thus the experiments were carried out in dogs with only one kidney and therefore constituted a more rigid test of the action of the alkali than was the case in previous studies. After operation the animals were placed on the usual stock diet; the fluid intake was not restricted. Four dogs (100, 30, 50 and 99) did not receive alkali and were killed (by intracardiac injection of air) after periods of fifty-eight, one hundred and seventy-nine, one hundred and eighty and three hundred and sixty-five days, respectively, to determine the effect of increased compensatory activity alone on the remaining kidney. This group, therefore, served as a further control for the entire series. Nine dogs received sodium bicarbonate in daily amounts which were gradually increased from 5 Gm. to 60 Gm. The smaller quantities of alkali were mixed with the food, while the larger ones were introduced via the stomach tube. Five dogs of this second group (47, 42, 88, 80 and 58) were given sodium bicarbonate by mouth for periods of only thirty to one hundred and fourteen days. The remaining four dogs (94, 66, 46 and 33) received sodium bicarbonate orally each day and intravenously each week for periods of one hundred and twenty-five to two hundred and sixty-one days. Dog 33 received the enormous quantity of 11,220 Gm. of sodium bicarbonate orally in two hundred and sixty-one days and twenty-one intravenous injections totaling 381 Gm., or 11,601 Gm. in all. The intravenous solution, consisting of 5 per cent sodium bicarbonate in distilled water, was warmed to body temperature and injected at the rate of 5 cc. per minute. Each dog gained weight during the experiment, the increases ranging from 2 to 4 Kg. The occurrence of convulsions during alkalosis usually indicated an irreversible acid-base disturbance, unaffected by intravenous administration of saline solution and resulting in death. All the animals except dogs 42 and 88

6. Suzuki, K.: Japanese J. M. Sc., Tr., IV, Biophysics **1**:67, 1927.

died in acute alkalosis. Autopsy was performed soon after death. The tissues were placed in Zenker-formaldehyde solution [7] and then were stained, after the usual preparation, by the so-called regressive hematoxylin and eosin stain.

RESULTS

Control Group.—No significant renal changes were observed after unilateral nephrectomy in the dogs not given sodium bicarbonate, as may be seen from the following analysis:

Dog 100 (wt. 12 Kg.). The control kidney (wt. 34 Gm.) was normal except for small mononuclear infiltrates near some glomeruli and surrounding the blood vessels between the cortex and medulla. A few casts were present in the lower collecting tubules.

The remaining kidney after fifty-eight days (wt. 36 Gm.) was slightly edematous. The tubules were separated and contained moderate amounts of fluid. The groundwork of many glomerular tufts was indistinct. Considerable desquamation of swollen epithelium was observed in some of the tubules. Areas of beginning focal necrosis were noted in occasional proximal convoluted tubules.

Dog 30 (wt. 7.8 Kg.). The control kidney (wt. 19.7 Gm.) was normal. The remaining kidney after one hundred and seventy-nine days (wt. 23.5 Gm.) was also normal except for a few small round cell infiltrates in the cortex.

Dog 50 (wt. 14 Kg.). Microscopic examination of the control kidney (wt. 36.5 Gm.) revealed marked focal pyelonephritis. Many of the glomerular loops were fused together and contained an increased number of cells. There were numerous foci of interstitial cellular infiltration in the medulla and a considerable degree of scarring throughout the kidney. Some glomeruli were hyalinized; others were atrophic, and the corresponding tubules were shrunken. There was mild leukocytic infiltration below the epithelium of the renal pelvis. The major arteries were normal.

The remaining kidney after one hundred and eighty days (wt. 35.2 Gm.) presented a similar appearance, although the inflammation was not as severe. Large areas of leukocytic infiltration extended from the cortex to the upper part of the medulla. There were many atrophic glomeruli and tubules.

Dog 99 (wt. 16 Kg.) (fig. 1 *A*). The control kidney (wt. 31 Gm.) was normal. The remaining kidney after three hundred and sixty-five days (wt. 32 Gm.) was also normal except for one old cyst in the medulla with surrounding leukocytic infiltration.

It will be noted that in these animals the removal of one kidney was not followed by compensatory hypertrophy of the other.

Group Given Sodium Bicarbonate by Mouth.—Minor changes appeared in the kidneys of the dogs given sodium bicarbonate by mouth only.

Dog 47 (wt. 8.9 Kg.). This animal was given 610 Gm. of sodium bicarbonate in thirty days. The control kidney (wt. 24 Gm.) presented evidence of chronic pyelonephritis. Tremendous subepithelial leukocytic infiltrates and many large areas of fibrosis extended through the cortex to the medulla. Many glomeruli

7. Zenker's solution prepared with solution of formaldehyde instead of glacial acetic acid (Helly's fluid).

were atrophic; the capsular spaces were distended and contained protein precipitate. The corresponding tubules were shrunken. The epithelium of the ascending limbs was vacuolated.

The "alkali kidney" (wt. 27.1 Gm.) was similarly involved, although the inflammation was less marked. There were many small glomeruli. The fibrosis and

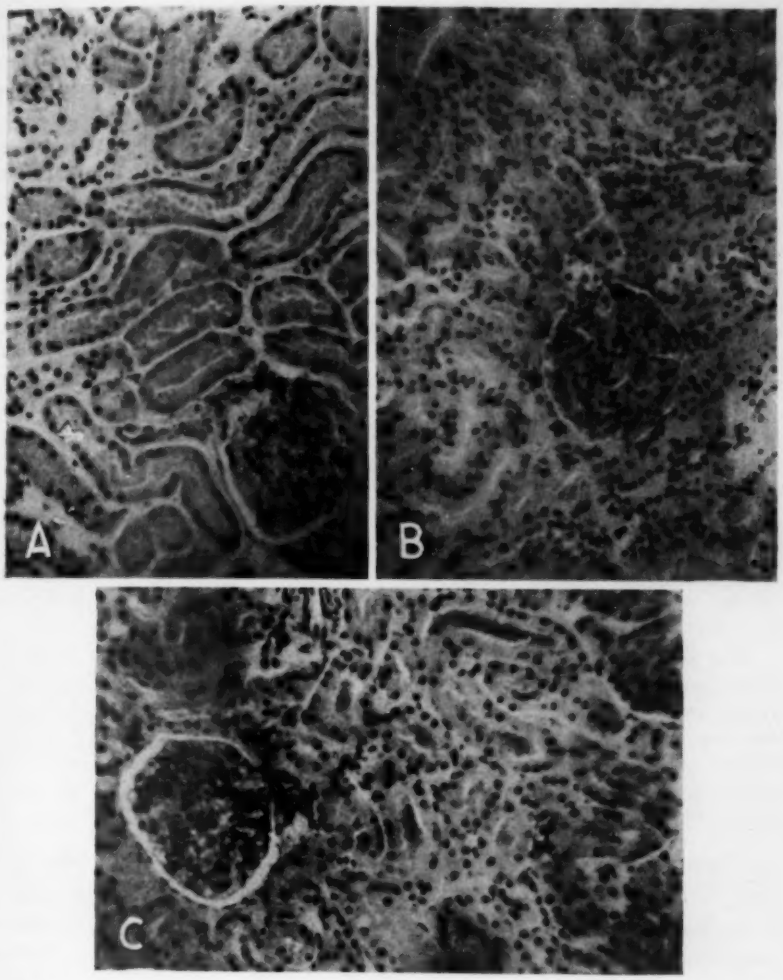

Fig. 1.—*A* (dog 99; no alkali), remaining kidney after three hundred and sixty-five days. Granular material may be seen in the tubules. × 250. *B* (dog 88), remaining kidney after 1,135 Gm. of sodium bicarbonate had been given in fifty-four days. A hyperemic glomerulus and normal tubules are seen. *C* (dog 46), remaining kidney after 6,789 Gm. of sodium bicarbonate had been given in one hundred and forty-nine days. The glomerulus is normal. Note the dense granular masses in collecting tubules. × 250.

scarring resembled those found in the control. In the upper part of the medulla, a number of the tubules were dilated and contained casts. In one area, the epithelium lining the tubules was necrotic. The pyelonephritis observed in the control kidney appeared to be less extensive after the ingestion of alkali.

Dog 42 (wt. 6.8 Kg.). This animal received 740 Gm. of sodium bicarbonate in forty-two days. The control kidney was normal (wt. 23 Gm.). The "alkali kidney" (wt. 29 Gm.) was moderately edematous. The glomeruli were normal. Protein precipitate was present in some of the tubules. The epithelium of the tubules in the inner cortex was mildly desquamated. A few masses of necrotic epithelium were noted in the upper part of the medulla. The only definite change, therefore, in the "alkali kidney" was the development of edema.

Dog 88 (wt. 9 Kg.). This animal was given 1,135 Gm. of sodium bicarbonate in fifty-four days (fig. 1*B*). The control kidney (wt. 25 Gm.) showed evidence of active filtration in that the glomerular spaces and tubules contained moderate amounts of fluid. Irregular blue-staining masses were present in some of the collecting tubules (probably necrotic epithelium). The glomeruli and tubules were normal.

The "alkali kidney" (wt. 26.2 Gm.) was hyperemic. A few of the glomeruli were hyalinized, but the vast majority showed no change. Granular dense blue-staining masses were observed in the lower collecting tubules, which were otherwise normal.

Dog. 80 (wt. 7.5 Kg.). This animal received 1,795 Gm. of sodium bicarbonate in sixty-six days. The control kidney (wt. 15.8 Gm.) showed evidence of previous inflammation. Occasional scars extended from the cortex to the medulla. There was perivascular cellular infiltration in the upper part of the medulla. Focal cellular infiltrates were noted in the lower part of the cortex and in the medulla. The glomeruli and tubules were normal.

The "alkali kidney" (wt. 15 Gm.) was less involved; there were only a few cellular infiltrates in the lower part of the cortex. The glomeruli and tubules were normal.

Dog 58 (wt. 6 Kg.). This animal was given 3,495 Gm. of sodium bicarbonate in one hundred and fourteen days. The control kidney (wt. 16 Gm.) was normal except for scattered areas of interstitial cellular infiltration in the lower part of the cortex and the upper part of the medulla.

The "alkali kidney" (wt. 20 Gm.) was congested. There were several small cellular infiltrates in the upper part of the medulla. The glomeruli and tubules were normal.

Group Given Sodium Bicarbonate by Mouth and by Vein.—The observations on the 4 dogs are of particular interest since, in addition to the daily oral administration of alkali, a weekly intravenous injection of sodium bicarbonate was given, producing moderately severe alkalosis.

Dog 94 (wt. 8.2 Kg.). This animal received 5,800 Gm. of sodium bicarbonate orally and 250 Gm. intravenously, the latter in eighteen divided weekly injections over a period of one hundred and twenty-five days. The control kidney (wt. 32 Gm.) was normal except for the presence of a few vacuolated cells in the epithelium lining the tubules in the lower part of the cortex.

The "alkali kidney" (wt. 34.5 Gm.) was moderately edematous, and protein precipitate was present in all the convoluted tubules. The glomeruli and tubules were normal.

Dog 66 (wt. 9.6 Kg.). This animal received 5,245 Gm. of sodium bicarbonate orally and 67 Gm. intravenously, the latter in four divided weekly injections over a period of one hundred and forty-nine days. The control kidney showed no changes except for the presence of large foam-shaped cells in the tubules. The remainder of the kidney was normal.

The "alkali kidney" (wt. 16 Gm.) was edematous. A protein precipitate was present in some of the convoluted tubules. In one area, many hyaline casts were

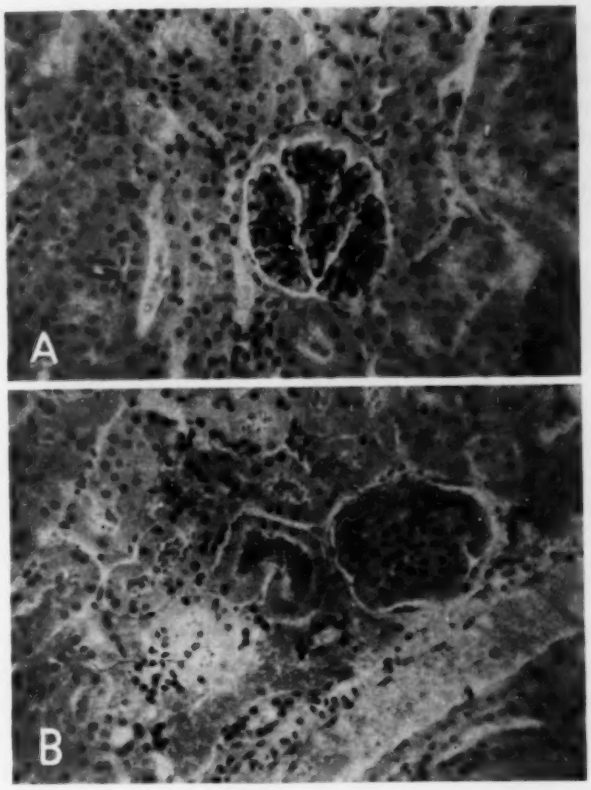

Fig. 2 (dog 33).—*A*, control. The glomerulus and tubules are normal. *B*, remaining kidney after 11,601 Gm. of sodium bicarbonate had been given in two hundred and sixty-one days. Note hyperemia of the glomerulus and dense granular masses in collecting tubules. The epithelium of the latter is partially desquamated.

seen in the tubules; the epithelium of the tubules, however, was normal. The glomeruli and major arteries were also normal.

Dog 46 (wt. 5.2 Kg.). The animal was given 6,530 Gm. of sodium bicarbonate orally and 259 Gm. intravenously, the latter in twenty divided weekly injections over a period of one hundred and forty-nine days (fig. 1 *C*). The control kidney (wt. 17.5 Gm.) was essentially normal. Some of the glomeruli and many of the tubules contained a fine precipitate.

In the "alkali kidney" (wt. 26.6 Gm.) the glomeruli and major arteries were normal. Numerous granular blue-staining masses were seen in the distal and collecting tubules. The tubular epithelium contained many dark nuclei, but the cell outlines were distinct. An attempt to stain the sections for calcium was unsuccessful because of the method of fixation. It was therefore impossible to decide whether these granular masses represented either necrotic epithelium or areas of calcification.

Dog 33 (wt. 10.7 Kg.). This animal received 11,220 Gm. of sodium bicarbonate orally and 381 Gm. intravenously, the latter in twenty-one divided weekly injections over a period of two hundred and sixty-one days (fig. 2). The control kidney (wt. 29.5 Kg.) was normal except for a few deeply staining masses in the tubules in the lower part of the medulla. A protein precipitate was present in a few of the glomerular and tubular spaces.

The "alkali kidney" (wt. 37.3 Kg.) was markedly hyperemic. There was focal scarring. An occasional glomerulus was hydronephrotic. The tubular capillaries were dilated, and many tubules contained a protein precipitate. There was moderate localized desquamation of the tubular epithelium. Many granular blue-staining masses were found in the collecting tubules.

Of interest in these dogs was the absence of inflammatory changes in the gastric mucosa. This finding is in contrast to the clinical belief that prolonged ingestion of alkalis results in gastritis.

COMMENT

Previous investigations have suggested that while albumin, casts or red blood cells may appear in the urine, no significant anatomic changes occur in the kidneys after the administration of sodium bicarbonate. Many of these experiments, however, especially those of Fischer, Kellert and Suzuki, are of little value in assessing the possible effects of the prolonged use of alkalis, since they consisted in the administration of a single large dose of sodium bicarbonate. The only long-continued administration of alkalis, except for the present study, was that carried out by Addis, MacKay and MacKay in rats, and although these investigators observed hematuria and hydronephrosis in some of their animals, the microscopic changes did not differ markedly from those of the control group. The present investigation differs from previous studies on three important points: For the first time dogs were employed as the test animals. Since the dog's kidney reacts functionally in a manner somewhat similar to that of the human kidney, the present experiments are perhaps more analogous to the problem in man. Secondly, control histologic examinations of the kidneys of the test animals were made possible by preliminary unilateral nephrectomy. The value of these control studies is demonstrated by the fact that in 2 dogs not given an alkali and in 4 which later received sodium bicarbonate the control kidneys showed evidence of previous injury—lesions which obviously could not be related to the effect of the alkali. The removal of one kidney also increased the excretory activity of the remaining

kidney and thereby provided a more rigid test of the effect of the alkali. Thirdly, large amounts of sodium bicarbonate were given orally and in 4 dogs also intravenously to insure the presence of continued alkalosis. In view of these stringent experimental conditions it is noteworthy that no marked anatomic renal changes were observed after the administration of sodium bicarbonate. Edema and hyperemia of the kidney were noted in 6 of the alkali-treated dogs. The edema was apparently caused by the fluids injected intravenously and also by the circulatory congestion associated with the convulsions appearing during the acute alkalosis. Moderate amounts of a protein precipitate were noted in the glomerular spaces and tubules of the kidneys of 3 dogs, suggesting that albumin may have been present in the urine during life. However, since both hyperemia and protein precipitate may appear very rapidly as acute antemortem changes, these observations cannot be considered indicative of chronic renal injury. Granular dense blue-staining masses were seen in the lumens of the collecting tubules in 3 dogs. A similar, although less marked, alteration was observed in the control kidneys of 2 of these animals. In the absence of specific calcium stains the differentiation of these masses either as necrotic epithelium or as foci of calcification was not possible. It seems likely, however, that the sodium bicarbonate was at least partially responsible for the appearance of these granular masses, since they were most numerous in the 2 dogs receiving the largest quantities of alkali (46 and 33). In dog 66 a few tubules contained many hyaline casts. In dog 33 the epithelium lining one section of tubules was moderately desquamated. This finding was the only sign suggestive of renal injury following the administration of sodium bicarbonate obtained in this study, but it was present to fully as great an extent in a control animal.

Of interest is the observation in 2 dogs that the pyelonephritis shown to be present by the control kidney appeared to be less extensive in the remaining kidney after the ingestion of sodium bicarbonate.

It is a well known fact that the functional behavior of the kidney often cannot be correlated with its anatomic appearance. It is possible, therefore, that clinical tests may indicate the presence of renal injury undetectable by present histologic methods. On the other hand, the results of the present study, demonstrating the absence of marked renal injury after the administration of sodium bicarbonate, are in accord with the recent clinical observation [8] that renal function impaired during alkalosis returns to normal or to its original level after the correction of the acid-base disturbance.

8. Kirsner, J. B., and Palmer, W. L.: J. A. M. A. **116**:384, 1941.

SUMMARY

Nine dogs were given large quantities of sodium bicarbonate daily by mouth for periods of thirty to two hundred and sixty-one days, and 4 of these animals were given the alkali intravenously at weekly intervals, to determine the effect of prolonged alkalosis on the structure of the kidney. The total amounts of sodium bicarbonate ingested ranged from 610 to 11,601 Gm. The study of each animal was controlled by a histologic examination of one kidney prior to the administration of the alkali and by similar examinations of the remaining kidneys of 4 dogs not given sodium bicarbonate but killed after periods of fifty-eight to three hundred and sixty-five days to ascertain the effect of increased compensatory activity alone on the kidney. The most frequent anatomic abnormalities observed in the "alkali kidneys" were hyperemia and edema, a protein precipitate in the tubules and, in 3 dogs, granular dense blue-staining masses in the lumens of the collecting tubules—all probably attributable to circulatory changes, alterations in the water balance and the precipitation of salts in the tubules secondary to the elevation of the urinary p_H. Hyaline casts were observed in the tubules of one dog, while in another the epithelium of one section of tubules was moderately desquamated. The only evidence suggestive of injury to the glomeruli or tubules was a localized desquamation of the tubular epithelium seen in one animal, but this was also observed in one of the controls.

CONCLUSION

The administration of large quantities of sodium bicarbonate by the oral and intravenous routes for periods as long as nine months does not produce marked or chronic anatomic change in the kidney of the dog.

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RESPONSE OF CARTILAGE AND BONE OF GROWING MICE TO TESTOSTERONE PROPIONATE

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In a recent article we analyzed the effect of an estrogen on the skeletal tissues of mice, rats and guinea pigs.[1] We shall now report on the action of an androgen on cartilage and bone of growing mice.

MATERIAL AND METHODS

Thirty-six virgin mice, 2 to 4 weeks old at the beginning of the experiment, received subcutaneous injections of 0.25 or 0.75 mg. of testosterone propionate (oreton) weekly for periods of: two weeks, and one, two, three, four, sixteen and nineteen months. Seventeen mice belonged to the closely inbred strain C_{57}, 15 to strain D and four to strain AKA. At autopsy, the long bones were removed for study, and dorsoventral sections of tibia and femur were prepared for microscopic examination, as described previously.[2]

MICROSCOPIC EXAMINATION (MICE OF STRAIN C_{57})

Epiphysial Line.—After administration of testosterone propionate for two weeks, in mice 6 weeks old the growth zone of the upper part of the tibia was distinctly narrowed. The cell count in a single cartilage cell row was 1 to 2 hypertrophic and 6 to 7 columnar cartilage cells instead of 4 hypertrophic and 10 columnar cartilage cells, as seen in untreated mice of corresponding age (fig. 1). The nonoriented cartilage cells were in a resting state and smaller than usual. The columnar cartilage cells had likewise decreased in size, and whereas in normal mice of this age numerous mitoses in these cells indicated

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The oreton used in these experiments was provided by Dr. E. Schwenk of the Schering Corporation.

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

Most of the material used in this investigation was secured from experiments conducted for other purposes by Dr. Leo Loeb.

These investigations were carried out with the aid of grants from the Committee on Research in Endocrinology of the National Research Council, from the International Cancer Research Foundation and from the Jane Coffin Childs Memorial Fund for Medical Research.

1. Silberberg, M., and Silberberg, R.: Am. J. Anat. **69**:2, 1941.
2. Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941.

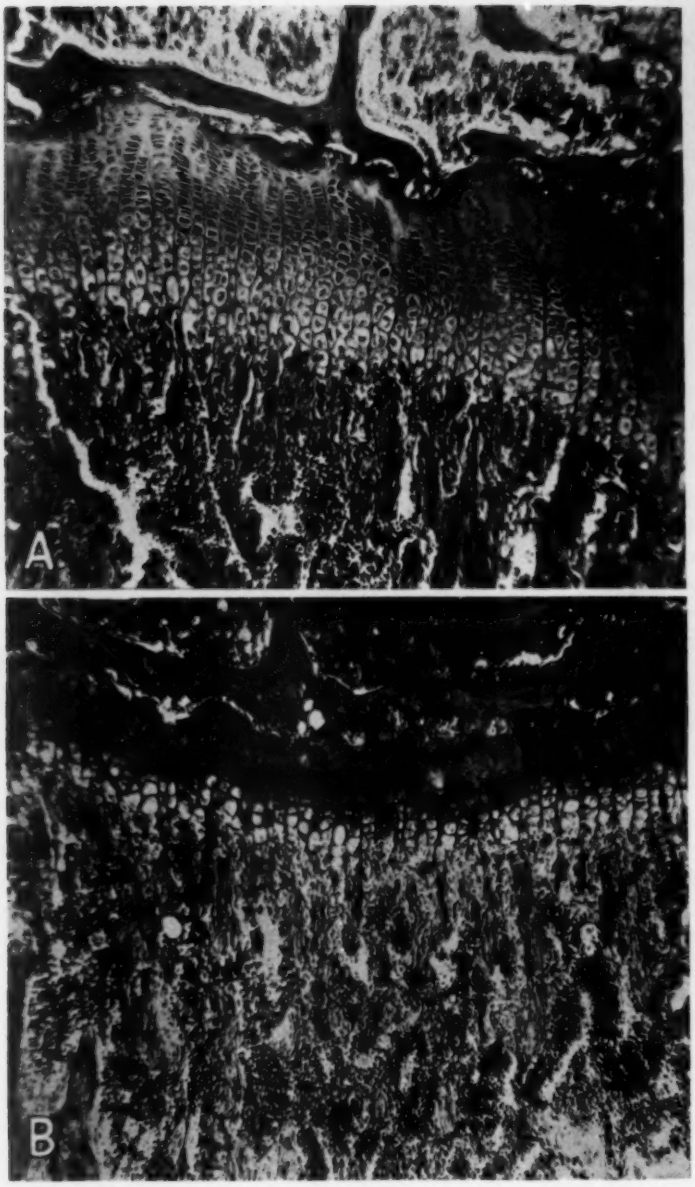

Fig. 1.—*A*, section through the upper part of the tibia of a normal female C_{57} mouse 6 weeks old. The epiphysial disk is wide, the cartilage cell rows have regular structure and the trabeculae are thin. Magnification, × 150. *B*, section through the upper part of the tibia of a female C_{57} mouse 6 weeks old which, starting at the age of 4 weeks, had received 0.25 mg. of testosterone propionate three times weekly for two weeks. Compare with *A*. The epiphysial disk is narrowed and more markedly calcified; the cartilage cell columns contain fewer and smaller cells than in normal mice of the same age; the subepiphysial trabeculae are more numerous and somewhat thicker. Magnification as in *A*.

strong proliferative activity, in the treated mice such proliferation was greatly diminished, mitoses being scarce or absent. Moreover, in contrast to the normal condition, there was no gradual transition of columnar into hypertrophic cartilage cells, an observation which points to an inhibition of the differentiation of the growing cartilage. The hypertrophic cartilage cells themselves were smaller than usual; their breakdown, induced by capillaries advancing from the bone marrow, was less active than in untreated animals. Instead of the ordinary process of replacement of the destroyed cartilage cells by bone, a direct metaplastic transformation of cartilage cells into osteocytes was noted here and there. The cartilaginous ground substance was increased in amount, dense, hyalinized or somewhat more heavily calcified than usual. In some places a sclerosed cartilaginous matrix encroached not only on the hypertrophic but also on the columnar cartilage cells. Single cartilage cells or single cartilage cell rows disintegrated or underwent hyalinization, and amorphous enclosures appeared in the epiphysial disk. In normal C_{57} mice, these changes were, as a rule, not observed before the age of 4 to 5 months.

After administration of testosterone propionate for four weeks, in mice 8 weeks old the epiphysial growth zone was still further narrowed. In the cartilaginous ground substance processes of hyalinization and calcification had made such progress that in some instances a cell count in the shortened cartilage cell rows could no longer be made. Where the cells could be counted, 1 to 2 hypertrophic and 4 to 5 columnar cartilage cells were present in a single cartilage cell row, as compared with the normal figures of 2 to 3 hypertrophic and 6 to 7 columnar cartilage cells in untreated mice of this age. Simultaneously, increased retrogressive changes had affected several adjoining cartilage cell columns, and hyalinized and ossified plugs had taken their place. Not infrequently, cartilage cells had been converted directly into bone cells, whereas the breakdown of the hypertrophic cartilage cells by capillaries and the subsequent replacement by bone were even more inhibited than after two weeks' administration of testosterone propionate.

In mice 2½ to 4 months old, which had been treated with testosterone propionate for two and three months, respectively, larger areas of the epiphysial plate had become ossified, and fairly thick bony plugs traversed the epiphysial disk in a vertical direction. The hyalinized matrix formed a transverse layer reaching into the zone of columnar cartilage cells and enclosing the atrophic cartilage cells (figs. 2 and 3*A*). In some instances, the bony plugs had been dissolved by elements of the bone marrow and had given way to perforations of the epiphysial disk. In normal C_{57} mice, a comparable condition had not been observed before the end of the first year of life.

After treatment with testosterone propionate for four months, the changes had not made much progress as compared with the preceding stage, but they were still advanced as compared with conditions in normal mice of corresponding age.

In mice 16½ to 19½ months old, which had received injections of testosterone propionate for sixteen and nineteen months, respectively, the epiphysial line was represented by a thin layer of sclerosed and hyalinized cartilage, poor in cells and containing only small amounts of calcium. The cartilage was walled off from both the epiphysial and the diaphysial marrow by an osseous plate. In several areas, ossification of the cartilage had made progress as compared with the earlier stages. In some cases, the perforations of the epiphysial disk had become wider than they had been at the earlier stages. But on the whole the

Figure 2

(See legend on opposite page)

condition of the epiphysial cartilage and the degree of epiphysiodiaphysial union reached under the influence of testosterone were similar to those seen in untreated mice of corresponding age.

The changes taking place in the epiphysial disk of the lower end of the femur followed a course not unlike that seen in the upper part of the tibia. In some instances, however, they were lagging slightly behind the latter.

Metaphysis and Diaphysis.—Whereas in normal young mice congested capillaries of the diaphysial bone marrow corrode the capsules of the hypertrophic cartilage cells, after two weeks of injections of testosterone propionate this was no longer the case—the vascularization of the subchondral zone was decreased. The metaphysial bone marrow was replaced by connective tissue which contained numerous epithelioid cells. Mitotic proliferation of these cells was slight, and osteoclastic giant cells were only rarely found. Most of the epithelioid cells were converted into osteocytes, particularly those lying close to the epiphysial cartilage and along the spurs of calcified cartilaginous matrix reaching from the epiphysial zone down into the metaphysis. The production of bony spicules took place in closer approximation to the epiphysial cartilage than usual. The spicules were more numerous, coarser and thicker than normal, and they adjoined the epiphysial cartilage with a broad base, while in untreated mice they were delicate in the metaphysis and become thickened step by step farther distally (fig. 1).

After one month of treatment with testosterone propionate, the aforementioned changes had become more accentuated. Osteoblasts had arranged themselves along the thickened spicules in even greater numbers than before, and gradually bony connections were produced, linking the spicules in a transverse direction. Some of the trabeculae contained short columns of preserved cartilage cells, as had been found after the administration of the estrogen. However, under the influence of testosterone, the increase in the amount of bone was less marked than under that of the estrogen and no dense interlaced bony network was formed, as had been the case when the latter substance was injected.[1]

After administration of testosterone propionate for two months, solution processes had led to thinning and shortening of some of the longitudinal spicules in mice then 2½ months old, but the remaining trabeculae were longer and thicker than normal for this age. Moreover,

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EXPLANATION OF FIGURE 2

Fig. 2.—*A*, section through the upper part of the tibia of a normal female C_{57} mouse 2½ months old. As compared with *A* in figure 1, the epiphysial disk is narrowed, the cartilage cell columns are shortened, the matrix is increased and the trabeculae are thicker. Same magnification as in figure 1. *B*, section through the upper part of the tibia of a female C_{57} mouse 2½ months old which, starting at the age of 2 weeks, had received 0.25 mg. of testosterone propionate once weekly for two months. Compare with *A*. The epiphysial disk is greatly narrowed; the cartilaginous matrix is greatly increased in amount; destroyed cartilage cell columns have been replaced by osseous plugs; subepiphysial spicules are thickened and in places are arranged in a transverse direction and linked with each other. Same magnification as in figure 1.

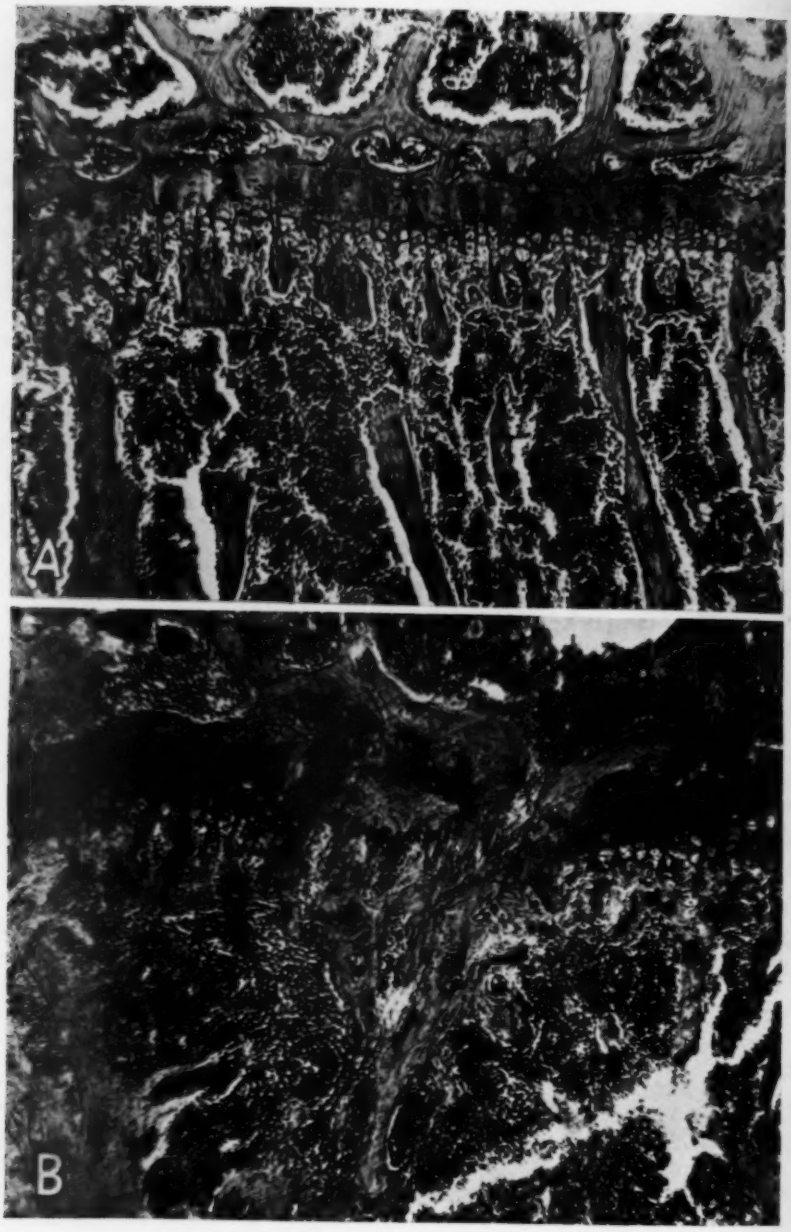

Figure 3

(See legend on opposite page)

a transverse osseous plate had begun to wall off the cartilaginous disk from the diaphysial bone marrow (figs. 2 *B* and 3 *A*). In untreated C_{57} mice, a transverse bony lamella was usually not seen before the age of 4 months, and then it was, as a rule, thinner than that in mice treated with testosterone propionate.

After administration of testosterone propionate for three and four months, congested capillaries corroded and thinned out the transverse osseous plate. Increasing processes of solution caused further thinning and shortening of the subepiphysial trabeculae.

With increasing duration of the experiment, the solution processes advanced and led to disappearance of the metaphysial spicules, and after sixteen and nineteen months of treatment the conditions resembled those seen in untreated mice of corresponding age.

The bone marrow in the diaphysis was cellular throughout the experimental period.

During the first two and three months of the injections, the compacta of the shaft was smooth and somewhat thickened, the osteocytes were fairly numerous but small and dense, the haversian canals were narrow, and larger amounts of calcified osseous ground substance were laid down than ordinarily. The periosteal tissue was somewhat thickened, and fairly numerous epithelioid cells were converted into bone cells, whereas processes of lacunar as well as of vascular absorption were less accentuated. In mice 16½ months old, treated for sixteen months, the compacta of the shaft was thinned out and softer than at the earlier stages and resembled that of normal old mice.

Joint.—After injections of testosterone propionate for a period up to two months, the cells of the sliding and transitional zones had a normal appearance, but ossification of the hypertrophic cartilage cells was farther advanced than is ordinarily the case at this age. After three months of treatment, slight proliferation and hypertrophy of the cartilage of the transitional zone were noted, but otherwise conditions

EXPLANATION OF FIGURE 3

Fig. 3.—*A*, section through the upper part of the tibia of a female C_{57} mouse 2½ months old which, starting at the age of 2 weeks, had received injections of 0.25 mg. of testosterone propionate three times weekly for two months. Conditions similar to those in figure 2 *B*. The cartilage cells and matrix are sclerosed. There is beginning solution of the subepiphysial trabeculae. Same magnification as in figure 1. *B*, section through the upper part of the tibia of a female D mouse 3 months old which, starting at the age of 4 weeks, had received 0.25 mg. of testosterone propionate three times weekly for two months. Cartilage of the epiphysial disk is being dissolved and replaced by bone and bone marrow. There is perforation of the epiphysial disk; otherwise conditions are similar to those described in *A*. Same magnification as in figure 1.

were about the same as at the earlier stages. In mice given injections continuously for sixteen and more months, processes of solution had led to thinning of the bony border lamella which delimits the articular surface from the epiphysial marrow. The cartilaginous ground substance was softened, and here and there slight proliferative changes, as well as processes of degeneration, such as karyolysis and karyorrhexis, were seen. These arthropathic changes, however, were less severe and less frequent than those in corresponding normal mice,[2] although they were somewhat more accentuated than those in mice which had received injections of the estrogen.[1]

STRAIN DIFFERENCES IN THE ACTION OF TESTOSTERONE ON THE SKELETAL TISSUES OF AKA AND D MICE

Under normal conditions, mice of strains C_{57} and AKA exhibit a slower rate of skeletal aging than mice of strain D. If the effect of testosterone on the skeleton of D mice is compared with that on the skeleton of C_{57} mice, these normal strain differences in the processes of aging must be taken into account. In 6 weeks old D mice which had been treated with testosterone for two weeks the cell count in the epiphysial cartilage of the upper part of the tibia was 2 hypertrophic and 7 columnar cartilage cells, as compared with 2 to 3 hypertrophic and 7 columnar cartilage cells in normal D mice of this age. This represents only a very slight change in the number of cells under the influence of testosterone, particularly if the corresponding figures for strain C_{57} are considered. These were 4 hypertrophic and 10 columnar cartilage cells for normal animals and 1 to 2 hypertrophic and 6 to 7 columnar cartilage cells for mice treated with testosterone propionate. Thus in strain C_{57} testosterone had caused a decrease of about one half to one third of the number of cells, whereas the number was scarcely changed in D mice. Degeneration of cartilage cell rows and formation of plugs were seen in testosterone-treated D mice at the age of 2 months, which is about one half to one month earlier than they were seen in normal D mice; in testosterone-treated C_{57} mice the same condition was observed also at the age of 2 months, but in normal C_{57} mice it is usually not present before the age of 4 to 5 months. In C_{57} mice the structural age of the cartilage was thus increased by testosterone by two to three months, whereas in D mice the testosterone raised the structural age of the cartilage by only one half to one month.

Solution processes were already advanced and had led to perforations in the epiphysial disk in 2½ and 3 month old D mice after two months of treatment with testosterone propionate (fig. 3 *B*), whereas the normal animal usually had reached the age of 6 to 7 months before a comparable condition had developed. In normal C_{57} mice, perforations

of the disk were encountered at the age of about 1 year; they were present at the age of 4 months after three months of treatment with testosterone propionate. If this stage of perforation is used as a standard, the structural age of the epiphysial disk of C57 mice was increased by about eight months by the testosterone, whereas that of D mice was advanced by only three and a half months as compared with the normal. As in strain C57, so also in strain D the effect of testosterone gradually receded, and in a D mouse 17½ months old which had been treated with testosterone propionate for seventeen months the appearance of the epiphysial disk was not different from that seen in untreated D mice of corresponding age.

COMMENT

In growing female mice testosterone accelerates the aging of the epiphysial cartilage: Proliferation of the resting and columnar cartilage cells and their differentiation into hypertrophic cartilage cells are inhibited; degeneration of cartilage cells and sclerosis, hyalinization and calcification of the cartilaginous matrix are intensified. Absorption of bone is inhibited during the early stages of the administration of testosterone propionate. Subsequently, the latter effect is reversed, and increased processes of solution cause a removal of the excess of bone and premature perforations of the epiphysial disk. These effects do not continue indefinitely but come to a standstill after a certain stage has been reached, and later the histologic picture resembles that seen in normal mice of the strain used, and the age changes in the cartilage, as a rule, do not exceed those seen in the normal old mice. High single doses of testosterone propionate administered over short periods of time are more effective in inducing the changes described than small single doses injected during a longer period.

As to the gross effects of testosterone on body growth, the conclusions of the various investigators differ. Levie [3] reported premature epiphysial closure and inhibition of growth in the tails of growing rats after two weeks of administration of testosterone propionate, but growth was less inhibited than after injections of an estrogen for a similar period. Rubinstein, Kurland and Goodwin [4] observed depression of body growth in growing rats from the fourth week on during treatment with testosterone propionate. Webster and Hoskins [5] could not confirm these findings in experiments on rats and rabbits.

3. Levie, L. H.: Acta brev. Neerland. **8**:53, 1938.

4. Rubinstein, H. S.; Kurland, A. A., and Goodwin, M.: Endocrinology **25**: 724, 1939.

5. Webster, B., and Hoskins, W.: Proc. Soc. Exper. Biol. & Med. **45**:72, 1940.

As far as the histogenetic mechanism is concerned, the effects of the androgen on cartilage and bone of growing mice resemble in certain respects those exerted on the skeleton by the estrogen, whereas in other respects the effects of the two substances on the skeleton differ. Both cause an acceleration of certain age changes in the epiphysial cartilage by inhibiting proliferation of the cartilage cells and by promoting degeneration, and hyalinization of the cartilage and sclerosis and calcification of the cartilaginous ground substance. Androgen and estrogen differ, however, in the degrees to which they influence the processes of absorption and of formation of bone. Normally, osteoblasts proliferate strongly, deposit bone and build up trabeculae, while absorptive processes, in which elements of the bone marrow participate, lead to gradual removal of the newly formed bony spicules.

Under the influence of estrogen, the proliferation of osteoblasts was greatly diminished, osteoclasts were scarce, and the vascularization of the subepiphysial tissue was decreased. The trabeculae were thickened, were more numerous and persisted for a considerable length of time. These findings were interpreted as the consequence of an inhibition of the aforementioned absorptive processes. These early changes were followed in the increased peritrabecular connective tissue by an accumulation of epithelioid cells, which took part in the production of the excess of bone seen during several months, after which time resorptive processes were resumed.

After administration of testosterone propionate, the proliferation of osteoblasts, the formation of osteoclasts and the vascularization of the subchondral tissue were likewise diminished as compared with the normal but to a lesser extent than in the case of estrogen. The spicules, although thicker and more numerous than normal, were thinner and less interlaced than in mice receiving estrogen, and they did not persist as long as under the influence of this substance. The latter findings suggest that resorptive processes were resumed as early as a few weeks subsequent to the injections of testosterone propionate. It may be concluded that (1) absorptive processes were less inhibited by testosterone than by the estrogen and (2) the bone formation was much less marked with the former than with the latter substance.

Furthermore, under the influence of testosterone, the articular cartilage was less hyalinized and the cartilaginous matrix remained softer than after administration of the estrogen. Therefore, in old mice treated with the androgen arthropathic changes were somewhat more severe and more frequent than in those treated with the estrogen, but their incidence and degree were still decreased as compared with conditions in normal old mice.

Under the influence of testosterone, aging processes in the epiphysial cartilage were more intensified in C_{57} mice, which normally show a

slow rate of skeletal aging, than in D mice, which ordinarily exhibit a fast rate of skeletal aging. In our report of a former investigation [1] we suggested that the lesser response of certain strains to the action of estrogen might be due to a better adaptation of these strains to that factor. It is, however, striking that these same strains should also possess, as we now find, a better adaptation to androgen and, as we observed previously, to anterior pituitary extracts and transplants.[6] Therefore it may be that besides the adaptation of the organism to the hormones, conditions in the tissues themselves set a limit to stimulation by these substances. This possibility is substantiated by the fact that the age changes of the skeletal tissues do not proceed beyond a certain maximum, even if androgen or estrogen is continuously administered for prolonged periods. Whenever this stage has been reached, no more effect is exerted by the administered substance. The cartilage of D mice may thus exhibit only a minor acceleration of skeletal aging under the influence of testosterone, whereas the cartilage of C_{57} mice, which ages slowly under normal conditions, may respond more strongly to the old age accelerating influence of this substance.

SUMMARY

Testosterone propionate promotes the aging of the epiphysial cartilage of growing female mice by inhibiting proliferative processes and, instead, promoting degeneration, hyalinization, sclerosis and calcification in the cartilage. It also inhibits for some time absorption of cartilage and bone, but to a lesser extent and for a shorter period than does estrogen. Hyalinization of the cartilage of the joint is less pronounced and arthropathic changes are, therefore, somewhat more frequent and severe under the influence of testosterone than after the administration of estrogen. The arthropathic changes, however, are decreased as compared with those in normal old mice. Under prolonged administration of testosterone propionate, the age changes do not progress beyond the maximum reached in normal old mice. The skeletal tissues of female C_{57} mice react more vigorously to testosterone than those of female D mice.

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6. Silberberg, M., and Silberberg, R.: Am. J. Path. **17**:189, 1941.

Forensic Medicine

LEGAL ASPECTS OF AUTOPSIES AND PATHOLOGIC SPECIMENS IN MISSOURI

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Medical jurisprudence as taught in various medical schools usually displays a uniformity that may be most misleading. Few members of the medical profession realize that each state has on its books laws[1] which in certain respects differ radically from the generally accepted opinions observed elsewhere. Since a previous communication,[2] certain facts have appeared which have forced a considerable change in attitude on this particular aspect of medicolegal subjects. A letter by Dr. W. C. Woodward[3] of the Bureau of Legal Medicine and Legislation of the American Medical Association is of greatest interest. This letter dealing with the legal aspects of the autopsy and tissue specimens brings to mind certain features of the laws of the state of Missouri relating to these and allied topics which are little known or realized even within the state of Missouri itself.

These points entail in part legal interpretations of the state laws as having a bearing on the departments of pathology of St. Joseph's Hospital, the Missouri Methodist Hospital and State Hospital No. 2 of St. Joseph, Mo. These departments are conducted in conformance with these legal interpretations as presented by the individual legal departments of the hospitals, the district attorney's office and the state's attorney general of the previous administration and, in the case of the powers of the coroner relating to autopsies, in conformity with several recent and older interpretations and judgments of the state's higher courts.

The legal restrictions surrounding the granting of permission to perform an autopsy are generally well known except in regard to the

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1. Dunn, H. L.: Physicians' Handbook on Birth and Death Registration, Containing International List of Causes of Death, ed. 9, United States Department of Commerce, Bureau of the Census, 1939, p. 2. Copies of this booklet are available at the offices of the Missouri State Board of Health, Jefferson City, Mo., and of local boards of health.

2. Hall, W. E. B.: J. Missouri M. A. **34**:15, 1937.

3. Woodward, W. C.: Am. J. M. Jurisp. **2**:299, 1939.

jurisdiction of the coroner's office. Contrary to generally accepted opinion, the office of coroner in Missouri is only quasijudicial. It does not carry with it unrestricted power to order an autopsy. This power is vested in the coroner's jury, or in the coroner if an inquest is to be held, or, under special circumstances and on appeal to that division, in the bureau of vital statistics as represented by the Missouri State Bureau of Health and then the medical officer of health of the local bureau or board of health. Autopsies ordered or granted by the coroner without such procedure, as they so often are today in hospitals in the case of unclaimed bodies, are illegal. When permission for an autopsy is so secured by a hospital, the hospital, the directorate, the superintendent, the intern, the pathologist and his assistant and the coroner are separately and collectively subject to suit.

The most recent decision bearing on this point was that by Justice Bland in the case of Patrick versus the Employers Mutual Liability Insurance Company ([Mo.], 118 S. W. [2d] 116).[4] An autopsy was performed on the body of a fireman employed by the city of Macon, Mo., without permission from the widow. Permission for an autopsy was granted by the coroner on the request of the defendant insurance company, which had insured the city against possible liability under the Missouri Workmen's Compensation Act. The autopsy was performed by a physician employed by the insurance company. Judgment for the widow was appealed to the Missouri State Court of Appeals, in Kansas City, Mo. The Court found that the widow's remedy for injury as incurred in the course of the unwarranted autopsy was not exclusively under the Workmen's Compensation Act. Rather it was instituted through a quasi property right[5] of the widow in the remains of her husband. The wrong she complained of occurred after the husband's death and was totally unconnected with the cause of death, and did not come under the jurisdiction of the compensation act. The autopsy, also, was illegally performed. In Missouri "a coroner has no authority to order an autopsy except in connection with an inquest to be held before a coroner's jury." In this case no inquest was held and none was intended by the coroner, as he had testified that the autopsy was performed solely to give him information on which to make out the death certificate (see later comment on this). Further, the defendant insurance company was not protected on the basis of the contention that if the coroner believed the autopsy was necessary he acted in a judicial capacity in having one performed. Instead, the court stated that the coroner acts judicially only in determining the necessity of an inquest and not in "the calling or holding of an autopsy." An autopsy may be ordered by the coroner only in connection with an inquest. Also, it could be concluded by the jury that, although the coroner sought to justify the autopsy on the grounds that it was necessary in order that he might sign the death certificate, he nevertheless abused his office in authorizing an autopsy the results of which might be used by the defendant insurance company in the securing of evidence in case of a claim for compensation to be made by the widow.

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4. Medicolegal Abstracts, J. A. M. A. **112**:2466, 1939.

5. For a general outline of the legal status of the body and of the obligations and rights of the estate and of the community the reader is referred to: Webster, R. W.: Legal Medicine and Toxicology, Philadelphia, W. B. Saunders Company, 1930, p. 81.

Another case came to my notice. A coroner had given permission for an autopsy to an intern—and hospital—to be performed on the body of a man who, demented by worries over drought, dust and fear of crop losses, had committed suicide by shooting himself in the head, after murdering his wife with his gun. His two brothers protested against the autopsy but it was performed by the assistant pathologist, who accepted the coroner's complete authority in the case, which had been one of violence. The two brothers, on behalf of the two children of the deceased man, then brought suit separately and collectively against the intern, hospital, assistant pathologist, chief pathologist and coroner. A settlement was made out of court. In the light of the previous court judgment the responsibility of the various parties is apparent.

In a case of death a physician must sign a death certificate,[5a] however brief his attendance, even when the cause of death is unknown, unless death was due to violence or the circumstances of death are sufficiently suspicious to warrant notification of the coroner. Refusal to sign makes the physician liable to a heavy fine and other punishment. Likewise, a coroner (see foregoing reference to this) must sign a death certificate when called to investigate a death, even though the causes of death are not apparent, unless, as pointed out before, the coroner's jury is called, in which case an autopsy may be ordered. Where the coroner's office is operated, as usual, on a limited budget, costly juries and autopsies are usually neglected, and since an autopsy alone may not be ordered, it is to be feared that many cases and crimes, which otherwise would be investigated, may go without proper scrutiny.

Unclaimed bodies are outside the coroner's jurisdiction unless violence is known or suspected. Autopsies on these are illegal; the bodies become the property of the Missouri State Anatomical Board, which should be notified at once. When the body is unclaimed by the board,[5] it is usually buried at the expense of the community in which death occurred or at that of the community where the deceased person had usually resided, if known. Although the local board of health and the local court may be considered to have ultimate jurisdiction in such cases, it might be considered unwise of hospital staffs to seek permission for autopsies on such bodies through these avenues. This statement is made in view of the fact that at any time a party showing a connection

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5a. This is in accordance with a decision of the Supreme Court of Missouri as given in an opinion by Judge Ernest S. Gantt on Nov. 18, 1929, regarding the making and signing of death certificates. The decision was rendered in the case of O'Donnell versus Wells (Mo., 21 [2d] S. W. 762). It was based on sections 5802 and 5803, Revised Statutes 1919, and on section 5817, Revised Statutes 1919, and chapter 48 (sections 11608 R. S. 1929—1164 R. S. 1929), Revised Statutes 1919. Simpson and others versus Wells, 292 Mo. 301, 237 S. W., cited by plaintiff, was ruled on the theory that the deputy coroner was an attending physician.

of association or relationship to the dead person might, by paying the expenses incurred by the community, lay claim to the body and its effects and subsequently institute suit against the hospital and the pathologist for an autopsy performed without his permission.

As to those hospitals which have looked to coroner's permits on unclaimed bodies to swell their autopsy percentages or which fear that loss of autopsies on unclaimed bodies, in coroner's cases, etc., may seriously affect their rating with the American Medical Association, I should like to refer them to the report [6] of the Council on Medical Education and Hospitals of the American Medical Association, which, under the heading "Computation of Necropsy Percentages," reported on the following resolution:

> RESOLVED, That those cases removed from the jurisdiction of a hospital by coroner or medical examiner, and in consequence not available as teaching material for interns, may be deducted from the total hospital deaths in computing hospital percentages. This provision also extends to bodies legally assigned to qualified educational institutions for dissection.

This chapter and the subsequent one, entitled "Revision of Necropsy Requirements," might well be reread with profit by all on a hospital staff who are even remotely connected with the functions of a department of pathology.

In Missouri, when an autopsy is performed, *all* organs must be returned to the body prior to completion of the autopsy. Only a sufficient amount of an organ may be removed and retained to allow for satisfactory microscopic study and diagnosis. Whole specimens as such must not be retained, nor are the pathologist and the hospital exempt from suit as violators of the law even when written consent of the nearest relative is obtained, regardless of the scientific importance of the specimen. Some pathologists propose to conform with this regulation by returning a small part of the organ to the body. Nevertheless, the interpretation has been given that museum specimens in the state of Missouri, unless derived from out of the state or as the result of surgical treatment, are prima facie evidence of law violation. This is a most unfortunate state of affairs, as many excellent and unusual specimens have been lost in this way, and the development of museums of pathology becomes slow and difficult.

However, a body, e. g., an embryo or a fetus, may be retained in a museum or a hospital if permission from the nearest relative is secured and if note is made on the death certificate of the location or disposal of the body. It might be stated that today Missouri, through the state board of health, in conformity with the United States Department of

6. J. A. M. A. **112**:922, 1939.

Commerce, Bureau of the Census,[7] requires registration of a stillbirth and of the birth of a fetus if the twentieth week of gestation has been reached. Also, Missouri now requires the use of the new "Standard Certificate of Stillbirth" as a combined birth and death certificate in the case of a stillbirth.[7] The state and federal bureaus of the census are concerned only with differentiation and certification of stillbirths and livebirths,[8] but from the medicolegal standpoint the definitions of such conditions differ, and the determinations of the result of a completed pregnancy as a viable child, a live one, a dead one or a stillborn one is of the greatest importance.[9] For this reason, every physician, whether within a hospital or outside in practice, should make it a habit, as a possible direct or indirect participant in some suit for damages, legitimacy, inheritance, patrimony, etc., to keep as a part of his personal records the various features of a delivery, miscarriage or abortion, including the location, date, duration of pregnancy as given by the mother, the male parent as claimed by her,[10] the sex of the fetus if apparent, and the measurements and external features of the fetus in order to give a relatively clear idea as to the true age of the child. With this there should be the record of whether the child was stillborn[9] or showed evidence of breathing, heart sounds, heart or cord pulsations or even the slightest voluntary movement at birth. Lastly, a duplicate of the birth certificate and of the death or stillbirth certificate should be kept, along with a note of having informed the mother or both parents as to the proper disposal of the body. When a hospital or a physician undertakes to dispose of a product of gestation,[1] even when the period of gestation is too short to require a death or a stillbirth certificate, it is imperative that these records be kept, together with the permission from the parents for such disposal and a note as to where and by what method the disposal was made. The general law is that any human body must be disposed of in a manner which will be generally acceptable and not repugnant to the community.[10a]

Here it may be stated that a hospital is directly responsible for the acts of its employees, including those of its interns. This comprehends a

7. Dunn,[1] p. 4.

8. Dunn,[1] pp. 2 and 4.

9. Webster,[5] pp. 210 and 230-250.

10. In Missouri the birth of a child born out of wedlock is legitimatized by subsequent marriage of the parents and acknowledgment or recognition by the father that the child is his. Marriage and acknowledgment that the husband is the father of the child are required of both parents only in Connecticut, Louisiana and New Hampshire.

10a. State [of Maine] versus Bradbury, 9 A. (2d) 657 (Maine 1939) and Reg. versus Price, 12 Q. B. D. 247. Reviewed in Medicolegal Abstracts, J. A. M. A. **116**:2527, 1941.

responsibility on the part of a hospital for the acts of any of its employees, professional or otherwise, who may be concerned with any of the functions of a department of pathology. Likewise, any physician is responsible for the acts of his assistants, medical or nonmedical, licensed or unlicensed, even if he is totally unaware at the time of such acts. However, none of this prevents suit from being brought against an assistant. These points are clear in the second case cited in this article. It might behoove every assistant, however temporary or permanent his position, to examine carefully his own medical liability insurance as well as that of his employer and arrive at a clear understanding of the full extent of his coverage, liabilities and responsibilities. In like manner, even the briefest courtesy participation by another physician in an autopsy may make a pathologist and his fellow physician co-defendants and mutually responsible for each other's performance.

The question of the ownership of surgical specimens does not appear to have been raised except for general acceptance that they are subject to claim by the patient, or in case of death, by the nearest relative. It is likewise accepted in the aforementioned departments that the patient may order or request that no histologic examination be made on his or her specimen. This point has been definitely settled in suits outside Missouri. No suit appears to have been instituted in the state on such a basis [11] but is possible as a form of assault and bodily violation. Otherwise it is taken as a privilege of usage for pathologists to perform whatever procedures are requisite for diagnosis or research. There appears to be no law regarding the disposal of unclaimed specimens except as their disposal may be required to meet the standard set for retention of specimens in hospitals approved by the American Medical Association as intern teaching institutions. Histologic and pathologic tissue sections are considered as the property of the hospital and its pathologist, as part of his records and as evidence required by him to arrive at a diagnosis. This point, too, has been verified by suit outside the state,[11] specimens taking a part and function identical with that of roentgen films.

The pathologist's surgical and autopsy reports are considered as "privileged communications," governed by the same rules as those of hospital reports and subject to the order of the court and the discretion of the patient or, in his stead, the nearest relatives. Of course, such

11. In legal practice, when a case in question is not covered by a definite state law or some prior judgment within the state courts, precedent and consideration are to be given to a judgment uttered in some similar case by a court in one of the other states of the Union or, occasionally, the courts of Canada and Great Britain. In view of this it is urgently suggested that readers become acquainted with: Medicolegal Cases, 1925-1930, Chicago, American Medical Association, 1932; Medicolegal Cases, 1931-1935, Chicago, American Medical Association, 1936; Weekly Medicolegal Abstracts, J. A. M. A., 1936.

reports must be identified by the pathologist, and, in the original, may be used by him as his notes to which he may refer in court.

Adequate medical liability insurance is a most wise investment, and conducive to real peace of mind. But a competent legal consultation [1] and a clear understanding of state laws in their bearing on the practice of medicine and the peculiar responsibilities of the various specialties may well afford the happiest guide in professional conduct.

SUMMARY

The office of the coroner in Missouri is quasijudicial and is greatly restricted in its powers and duties relating to autopsies, inquests and the signing of death certificates. The coroner may order an autopsy only when there is suspicion of violence or death by unlawful means and when an inquest is going to be held.

The duties of an attending physician or of a coroner with regard to death certificates are outlined.

Unclaimed bodies are subject to the jurisdiction of the Missouri State Anatomical Board, a local court or, in certain cases, the Missouri State Board of Health, and not to that of the coroner except in circumstances in which an inquest will be called.

In Missouri all of an organ must be returned to the body after an autopsy, except for a specimen sufficient to allow satisfactory microscopic study and diagnosis.

Certain privileges and precautions are to be observed in cases of birth, miscarriage or abortion.

Surgical specimens have been accepted as subject to claim by the patient or the nearest relatives, but microscopic tissues and surgical and autopsy reports are part of the hospital's and pathologist's records and are "privileged communications."

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Case Reports

OSTEOGENIC SARCOMA OF MENINGEAL ORIGIN

Report of a Case of Meningeal Tumor with Both Osteoblastic and Osteoclastic Activity

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Meningioma containing bone within its substance is not particularly common, although by no means rare. Among the 313 verified examples of meningioma which form the basis for the monograph by Cushing and Eisenhardt,[1] 6 of the type concerned here were recorded and placed in a separate category. The literature contains scattered accounts of such tumors, but from the descriptions given it is at times difficult to distinguish primary osseous tissue within the neoplasm from the overgrowth (hyperostosis) of the overlying calvarium caused by certain meningeal tumors. Such overgrowth, which presumably is due to infiltration of the bone by tumor tissue, has been the subject of considerable discussion but is not related to the subject of the present paper.[2]

The bone within meningioma differs in certain characteristics from the bone produced by invasion of adjacent osseous structures or by incorporation of such structures in a neoplasm. In the former instance the bone lies well within the substance of the tumor, away from the dura and removed from endosteal projections of the inner table of the skull. Further, the osseous tissue in meningioma occurs without the presence of hyperostosis of the calvarium, as was the case with the growths removed by Cushing. In general, meningioma containing bone is a slowly growing and more or less indolent growth, unlike certain of the related chondroblastic tumors, in which sarcomatous transformation may take place. Such a tumor which showed diffuse infiltration of the brain was described by Wolf and Echlin.[3]

REPORT OF CASE

A woman 37 years old was admitted to the Mayo Clinic in July 1937, with the complaints of weakness of the right hand, convulsive seizures and inability to speak. About three to four months before admission, after reading for several hours, she suddenly felt a severe crampy pain in the right arm, which was associated with a feeling of fear and was followed by unconsciousness for about

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1. Cushing, H., and Eisenhardt, L.: Meningiomas: Their Classification, Regional Behaviour, Life History, and Surgical End Results, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

2. Cushing, H.: Arch. Neurol. & Psychiat. **8**:139, 1922. Spiller, W. G.: ibid. **21**:637, 1929. Phemister, D. B.: Arch. Surg. **6**:554, 1923. Alpers, B. J., and Harrow, R.: Arch. Neurol. & Psychiat. **28**:339, 1932.

3. Wolf, A., and Echlin, F.: Bull. Neurol. Inst. New York **5**:515, 1936.

fifteen to twenty minutes. At this time she apparently had a convulsion on the right side, which left her with marked weakness of the right arm. Strength returned gradually. The patient had become irritable and nervous and in the past year had had progressive oligomenorrhea. About a month after the first convulsion, a second similar episode occurred and she was unconscious for about forty-five minutes. Complete paralysis of the upper right extremity ensued. In the two months preceding admission to the clinic and hospitalization, four to five such convulsive seizures had occurred, but she had not lost consciousness. Tonic and clonic movements of the right arm had occurred for about two to three minutes, and with each episode the weakness of the right upper extremity became more marked and the return of function less evident. Preceding each attack by about fifteen seconds was the crampy pain in the right arm as well as the sensation of fear. The convulsive movements involved first the arm, then the leg and finally the face, and were confined to the right side of the body. For about one month prior to admission she had noted some weakness of the right leg, which had become progressively worse, and for two weeks before hospitalization she had been unable to walk.

There had been some speech difficulty from the onset of the illness, and for six weeks the speech had been slurred and scanning, with marked hesitancy, characteristic of motor impairment. For a similar length of time occasional paresthesias in the form of tingling involved the right arm at first and later the right leg. Unsteadiness had been noted about two months prior to the time she became bedridden. Headache had not been a prominent part of the illness but had developed shortly before admission; it centered in the left parietal region. There had been no vomiting and no dizziness. The patient was right handed.

General examination at the time of admission disclosed nothing unusual aside from the neurologic findings. The patient was bedridden and appeared to be somewhat confused. Speech was halting and slurred; marked but incomplete motor or expressive aphasia was present. The hemiplegia was more marked in the right upper than in the right lower extremity. Aside from the presence of right facial weakness, supranuclear in type, the cranial nerves were intact. Vision was impaired, owing to a high grade of myopia, but the fundi were not unusual, and there was no papilledema. The visual fields were normal when tested with the tangent screen and were normal aside from a relative right homonymous contraction when tested with the perimeter. Astereognosis, adiadokokinesia and poor coordination were marked on the right side. There was right hyperreflexia with absence of abdominal reflexes and a strongly positive right extensor Babinski sign. The Oppenheim sign was slightly positive on the right, and ankle clonus was present and well sustained on this side.

Roentgenographic examination of the skull disclosed an area of erosion 2 cm. in diameter in the superior portion of the left parietal bone. The borders appeared irregular and moth eaten. The thorax and long bones disclosed no evidence of a metastatic lesion.

The preoperative diagnosis was tumor involving the left frontoparietal region.

Operation.—With the patient under intratracheal nitrogen monoxide and ether anesthesia, a large osteoplastic flap was reflected, centering about the eroded portion of the skull but including the frontal as well as the parietal region. All the tissues were vascular and hemorrhage was controlled with difficulty. The tumor was found to have invaded the dura and to have caused considerable erosion of the skull. Incision of the dura disclosed a large, well encapsulated tumor in the postfrontal and parietal region; a small portion was attached to the dura near the vertex. The attached dura was removed with the growth, the tumor being rolled out of its bed of compressed brain tissue after the vascular attachments had

been controlled. The main hemorrhage was found to originate from a large communicating vein draining into the longitudinal sinus. As far as could be seen grossly, the tumor was completely removed. The extreme vascularity necessitated two transfusions of blood of 500 cc. each during the operation.

Postoperative Course.—Aside from some elevation of temperature during the first three days, the postoperative course was not unusual, and the wound healed by first intention. Voluntary movements of the right arm improved steadily and were almost normal at the time of dismissal, on the fifteenth postoperative day. The aphasia did not improve until the sixth day, but after that there was steady progress toward normal speech. In the course of the convalescence there were frequent episodes of numbness and tingling of the right upper extremity. Stereognosis and coordination were still impaired at the time of dismissal.

After the patient's return home the progress continued, and when she was seen at the clinic ten months after operation objective examination disclosed little and there was no evidence of recurrence of the growth. The patient experienced several episodes of jacksonian convulsions, which were partially controlled by the use of phenobarbital. Correspondence with the family physician in 1939, two years after operation, disclosed that the patient had suffered from mental depression but that hospitalization and spinal puncture gave no evidence of recurrence of the tumor.

Pathologic Characteristics.—Grossly, the tumor measured 6 by 4 by 3 cm. and appeared well encapsulated except for a portion measuring about 1 cm. in diameter where it had invaded and penetrated the dura. A small nubbin of tumor tissue projected 0.5 cm. above the outer leaf of the dura, and in this region the central core of the tumor was occupied by a spicule of bone. The tumor tissue was grayish yellow in the fixed specimen and was firm but compressible. Aside from the spicule of bone, no other gross bone or calcification was present. The outer surface of the tumor was smooth and without nodules, and several large vessels could be identified in the capsule The cut surface was smooth and without visible whorls; numerous large vessels, some of which measured 4 to 5 mm. in diameter, were present. No cysts were evident.

Microscopically, the tumor was enclosed in a loosely woven capsule of collagenous connective tissue which contained numerous, moderate-sized blood vessels. The line of demarcation between tumor and capsule was not sharp and there was no subcapsular layer of compressed tumor tissue.

The microscopic appearance of the tumor was striking and in many respects bore a close resemblance to the primordium of the parietal bone of the 4 month embryo depicted by Schaffer.[4] The tumor was highly cellular and was composed essentially of two types of cells, morphologically different but developmentally and physiologically closely related (fig. 1 *a* and *b*). Scattered throughout all parts of the tumor were foci of newly formed bone and of osseous tissue in all stages of formation. Closely related to these, and arranged in more or less radial fashion about the surface, was a layer of large multinucleated cells. These varied considerably in size and had abundant granular cytoplasm, which was slightly basophilic and usually contained two or more large nuclei. The latter had coarse, deeply staining chromatin material and several nucleoli. In some places the cells related to the surface of the developing bone were smaller and contained only one eccentrically placed nucleus, although the cytoplasm was similar to that of the giant cells. These cells in general were disposed in shallow and deep excavations

4. Schaffer, cited by Maximow and Bloom.[5]

in the scalloped edge of the developing osseous tissue and frequently were enclosed in the lacunae of the newly formed bone. Other giant cells, even larger than those just mentioned, could be identified as osteoclasts (fig. 2*a* and *b*).

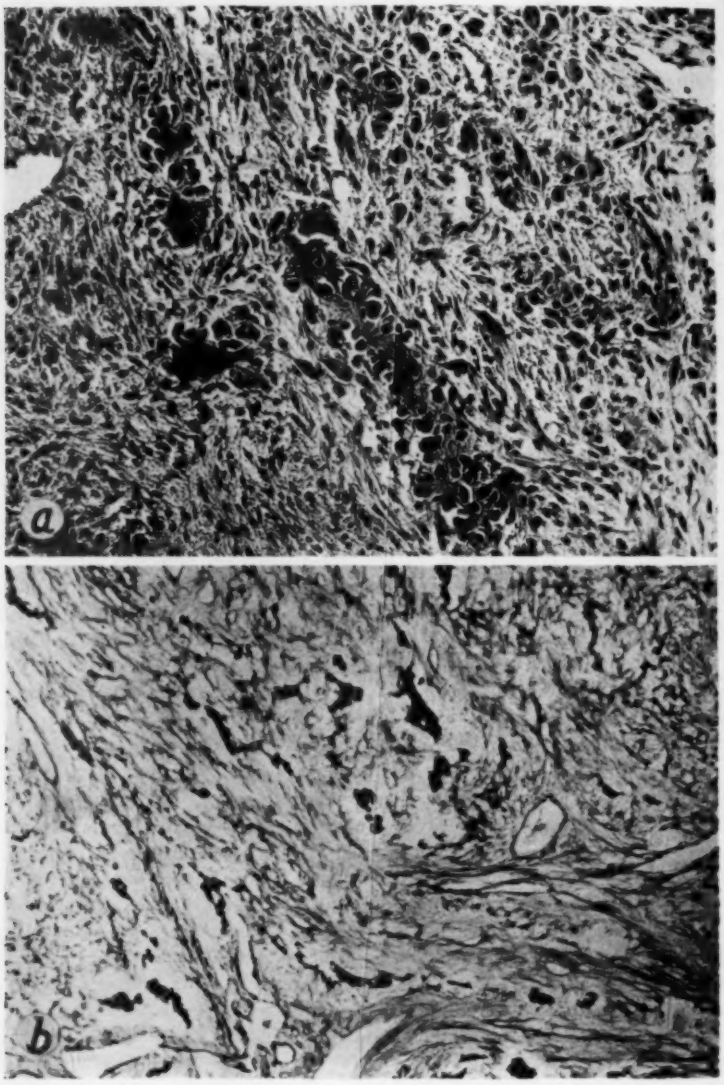

Fig. 1.—The tumor: (*a*) foci of bone formation and the fibroblastic tissue which intervenes (hematoxylin and eosin; × 140); (*b*) section stained to show the character of the stroma. The tendency for some streaming between the heavily impregnated foci, which represent bone, can be noted (Perdrau; × 65).

Between the foci of bone formation bundles of typically fibroblastic tissue passed in close relationship to the surface layer of the osteoblastic cells. This tissue was composed of elongated cells with bipolar distribution of acidophilic cytoplasm and elongated and somewhat chromophilic nuclei. Small portions of

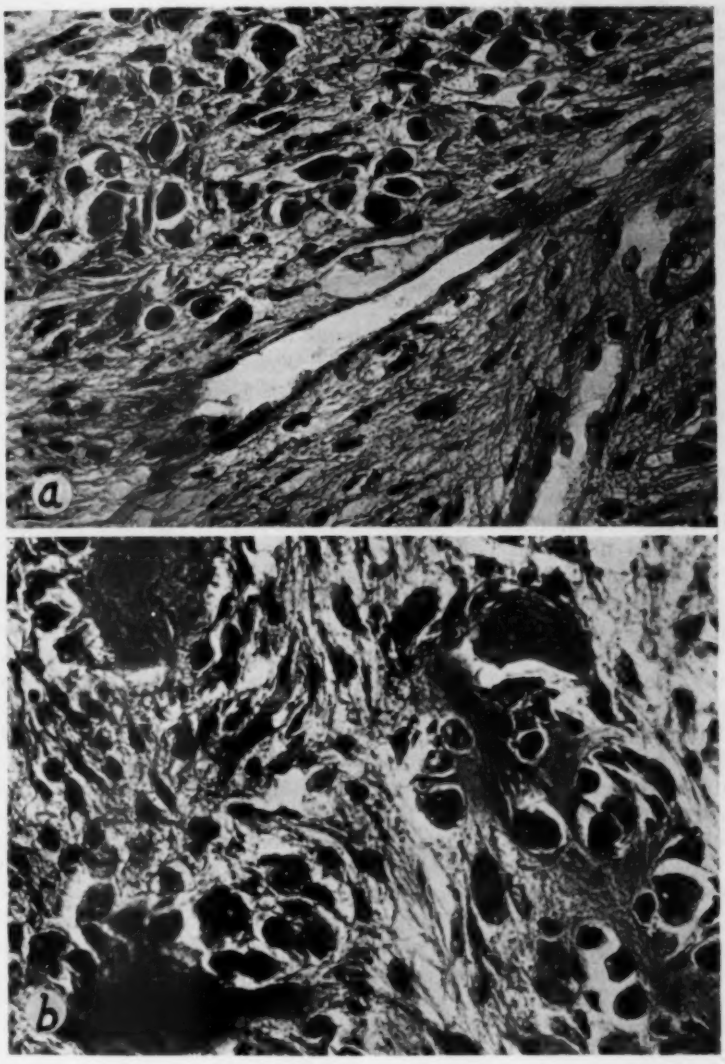

Fig. 2.—Character of the tumor in (*a*) an area in which giant cells predominate and there is little evidence of formation of bone (hematoxylin and eosin; × 300) and (*b*) the central portion of the tissue shown in 1*a*. The large multinucleated cell adjacent to the spicule of bone is an osteoclast, and the dark mass in the lower corner represents calcification in the process of the formation of osseous tissue (hematoxylin and eosin; × 400).

the tumor appeared to be composed predominantly of the large giant and multinucleated cells, with little of the osseous tissue present, while in other portions the fibroblastic elements were in preponderance. In general, however, the structure of the tumor was constant.

The Mallory differential stain revealed no difference in staining properties between the essentially fibroblastic foci and the other portions of the tissue. No glial fibers could be identified with appropriate stains. Mitotic figures were encountered frequently and were confined, for the most part, to the fibroblastic elements. This is interesting in view of the statement of Maximow [5] that mitotic division of the osteoblasts is seen seldom in developing bone. The tumor contained moderate-sized blood vessels with poorly formed walls. There was considerable hemorrhage of recent origin in some portions of the tissue, but degenerative changes were at a minimum. Evidence of chondroblastic activity was absent, and the bone formed was of the membranous type. True whorl formation and psammoma bodies were not present.

COMMENT

The presence or formation of bone within the dura mater or the arachnoid is not unusual, and when it occurs within the former it is commonly in the region of the falx cerebri. The nature and origin of these osseous deposits, however, are not understood fully and have been the subject of some controversy. Cleland,[6] in an early description of two meningeal tumors in which bone was present, maintained that the bone arose from arachnoidal cells which had invaded the dura mater. Cushing and Weed [7] demonstrated that bony plaques were not infrequently encountered in the arachnoid. In a recent review of the subject of calcification and ossification in the meninges, Halstead and Christopher [8] expressed the view that aside from ossification secondary to an inflammatory process, the presence of bone in the meninges may be accounted for by the retention of the osteogenic function in islands of cells composing the dura mater. They made no statement with regard to the origin of bone in meningeal tumors.

Focal ossification is frequently seen in almost all organs of the body and in a great majority of the various forms and types of tumors. It can occur wherever calcium is present and is dependent on the acquisition of osteoblastic properties by fibroblasts. Ewing [9] listed four factors which, in general, may be regarded as tending to influence or call forth the osteoblastic properties of fibroblasts: (1) proximity to bone; (2) the presence of calcific deposits, for in many instances this figures in ossification of necrotic tissues; (3) the presence of an active productive inflammatory process, and (4) in certain instances a special predisposition to calcification and ossification, possibly related to disturbances of calcium metabolism. In many instances the formation of heteroplastic bone occurs without the appearance of many osteoblasts; the fixed cells are

5. Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938, pp. 128-131.

6. Cleland, J.: Glasgow M. J. **11**:148, 1863-1864.

7. Cushing, H., and Weed, L. H.: Bull. Johns Hopkins Hosp. **26**:367, 1915.

8. Halstead, A. E., and Christopher, F.: Arch. Surg. **6**:847, 1923.

9. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 218.

incorporated passively in the osteoid and osseous matrix. This combination of factors may account to a large part for the presence of bone in certain of the meningeal tumors.

With the exception of the formation of bone and the "metastatic calcification" of Virchow, calcification seldom occurs in normal tissue, some form of degeneration being required before deposition of lime salts occurs. Wells [10] gave an account of the chemistry and other factors involved. Although calcification may occur in any sort of cell or tissue, provided the process of degeneration or devitalization has progressed sufficiently, ossification is accomplished only in varieties of connective tissue. Calcification occurring in epithelial cells is preceded by hyaline changes, and it is in this hyaline substance that the calcium is deposited. Further, any region of calcification may be replaced by bone, regardless of the tissue involved, and the presence of calcific deposits in any part of the body appears to stimulate the connective tissue to form bone.[11]

Calcification is one of the most commonly encountered forms of retrogressive change in meningeal tumors. Formation of bone, however, is not seen frequently. In a review of 60 spinal meningeal tumors seen in the Mayo Clinic, in 1927, Learmonth [12] made no mention of the presence of osseous tissue, although he stated that in the majority of the older tumors diagnosed as leptomeningioma, microscopic examination disclosed varying degrees of calcification. Brown,[13] however, in a recent review of 130 spinal meningeal tumors at the clinic encountered 4 in which osseous tissue was present, and in some of these 4 tumors definite evidence of osteoblastic activity was found. Weiser,[14] according to Cushing and Eisenhardt,[1] was the first clearly to distinguish between the hyperostosing effect of the dural endothelioma and the formation of true bone within its substance. Bailey and Bucy,[15] in their classification of meningeal tumors, placed these bone-containing tumors in a separate category and termed them "osteoblastic meningiomas." They described an intraspinal tumor which was situated entirely outside of the arachnoid membrane and which was ossified so completely that it cast a heavy shadow on roentgenographic examination. This tumor had been present for at least three years prior to operation. According to Bailey and Bucy, the bone formed in these tumors is always of the membranous type. In their tumor there were bands of osseous tissue with nonosseous tissue appearing as a sort of fibrous marrow. Calcified psammoma bodies were present, but no mention was made of the presence of osteoblasts or of osteoblastic activity. The nonosseous tissue was composed of elongated cells with nuclei of the connective tissue type. Microscopic examination of the tumor of the cord described by Rogers [16] disclosed numerous psammoma bodies lying in a fibrocellular matrix in which

10. Wells, H. G.: Chemical Pathology: Being a Discussion of General Pathology from the Standpoint of the Chemical Processes Involved, ed. 4, Philadelphia, W. B. Saunders Company, 1920.

11. Nicholson, G. W.: J. Path. & Bact. **21**:287, 1917.

12. Learmonth, J. R.: Brit. J. Surg. **14**:397, 1927.

13. Brown, M. H.: Unpublished data.

14. Weiser, A.: Deutsche Ztschr. f. Chir. **192**:405, 1925.

15. Bailey, P., and Bucy, P. C.: Am. J. Cancer **15**:15, 1931.

16. Rogers, L.: Brit. J. Surg. **15**:675, 1928.

large masses of bone were present. Again, no evidence of an active osteogenic process was evident. This, then, appears to be an example of the formation of vicarious or heteroplastic bone in a tumor containing much calcium.

Evidence of an active osteogenic process has been described in only a few of the meningeal tumors containing bone. In the examples of Bailey and Bucy [15] and of Rogers,[16] along with others reported in the literature, the bone present appears to have been merely heteroplastic osseous tissue, the formation of which had been stimulated by the presence of calcium over a long period and not, as has been suggested, by an inherent osteogenic property of the parenchyma of the tumor. On this basis, then, an explanation must be given for the frequency with which calcification is found and the infrequent appearance of bone in these meningeal growths. Ossification apparently occurs only in the presence of an optimal chemical and morphologic environment. Extreme calcification in tumors, aptly described by Bland-Sutton as "dead tumors," implies extensive damage and diminished viability of the growth, a condition not compatible with the metaplastic process which converts the fibroblasts to osteogenic activity. Ossification occurs through a uniform distribution of lime salts in a ground substance. Psammoma accounts for a large proportion of the calcification in the meningioma. Psammoma, which apparently has some central body as a nidus, a small vessel or a collagen fiber, represents a type of calcification, more closely related to a concretion than to that which may give rise to formation of bone. Another factor involved is time. Ossification, even in the presence of optimal factors, proceeds slowly, and a review of the literature suggests that tumors which contained bone had been present for a longer period than is usual. The severe symptoms which arise from compression of the nervous tissue result in removal of the tumor or in death before ossification can take place in the majority of instances.

In the case presented herein, active osteogenic and osteoclastic processes were evident throughout the entire growth, and osseous tissue in all stages of formation could be identified. In no place did examination reveal bone without evidence of cellular activity about it. The large size of the tumor, the evidence of invasion of the dura and the short clinical history suggest a highly active or malignant growth, particularly in view of its location over a portion of the cortex which is prone to give early signs of compression. The sarcomatous character of the fibroblastic elements accounts for the rapid growth and relatively large size of the mass. Despite the inclusion of a small spicule of bone at the point of erosion of the inner table of the skull, nothing in the microscopic appearance was suggestive of origin from the calvarium. The sections of tissue from the portion of the tumor remote from the dural attachment were no different on examination from those taken close to the point of invasion. Otherwise little can be said of the probable origin of the tumor. Despite the fact that cells similar to the arachnoidal elements could not be identified, the tumor probably arose from either the arachnoid or the dura.

SUMMARY

An unusual intracranial tumor of meningeal origin has been described, in which active osteogenic and osteoclastic processes occurred

throughout the tissue in combination with sarcomatous transformation of the intervening fibroblastic connective tissue.

From a study of this tumor and from a review of the literature, it has been suggested that true osteoblastic meningeal tumors are rare and that the osseous tissue in most of the so-called osteogenic tumors diagnosed as meningioma is an example of heteroplastic or vicarious formation of bone, secondary to the changes which cause ossification in other organs and tumors in the body.

The criterion for the osteogenic character of these meningeal tumors should be the presence of an active cellular osteoblastic process rather than the mere inclusion of bone in the tumor tissue.

LEPROSY WITH WIDESPREAD TUMOR-LIKE TUBERCULOSIS

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The complication of chronic leprosy by tuberculosis is frequent. Not only do the general conditions of life of the patient with leprosy favor development of tuberculosis on exposure to infection with the tubercle bacillus, but also there may be relations of allergy between the two diseases. Bieling and Schwartz [1] obtained allergic reactions in guinea pigs with bovine tubercle bacilli after previous injection of lepra bacilli. As this question has not yet been answered satisfactorily and, on the other hand, the aspect of tuberculosis in the present case is rather extraordinary, a report of the case may be of general interest.

REPORT OF A CASE

A man of Indian race, 44 years old, had for five years eruptions on the face, which were diagnosed as tuberculous leprosy of the face, ears and nose. Two years before the beginning of the eruptions he had stayed some time in a place where leprosy is frequent. Recently he had lost weight and suffered from cough. He became hoarse and at last aphonic. He had diarrhea. Because of a backache he could not sit up. He died with increasing weakness, dyspnea and fever.

Autopsy (six hours after death).—The body was 150 cm. long and rather emaciated. The eyebrows were missing. Papular efflorescences in groups were apparent in the regions of the eyebrows, on the cheeks, on the extremity of the nose and on the left ear. They were covered partially with crusts; partially they showed irregular surfaces. There was edema of both feet and of the scrotum.

Both pleural cavities contained a clear, somewhat hemorrhagic fluid, 300 cc. on the left and 100 cc. on the right. The pleuras were normal except for a small opaque zone on the inferior lobe of the left lung. Both lungs showed edema. In all parts of the lungs there was a dense dissemination of miliary tubercles, some of them with central caseation. In the inferior lobe of the left lung there were two small areas of bronchopneumonia. Purulent liquid appeared in the sections of the bronchi.

The pericardial sac contained 20 cc. of a clear yellowish fluid. The heart was slightly dilated on both sides, the muscle flabby. The valves and great arteries were normal.

The epiglottis was thickened, deformed and soft. The mucosa of the larynx and trachea, especially that of the false vocal chords, was thickened and of a grayish color. In the bifurcation of the trachea were some lymph nodes with small tubercles.

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Part of the microscopic work and the figures have been executed in the Pathological Institute of the University of Concepción, Chile (Prof. Dr. Ernesto Herzog, director).

1. Bieling and Schwartz: Verhandl. d. deutsch. path. Gesellsch. **25**:347, 1930.

The peritoneal cavity contained about 1,000 cc. of a clear yellowish fluid. The liver was much enlarged, especially in the right lobe; it measured 28 by 20 by 11 cm. The surface showed many prominent yellow nodules. On the cut surface there were numerous round areas of caseation, some of them polycyclic, ranging up to 7 cm. in diameter. These areas were separated from hepatic parenchyma by a small grayish zone. There was congestion of the central parts of the lobules (fig., *A*). The gallbladder and bile ducts were normal. In the porta of the liver were lymph nodes of the size of pigeon's eggs, with areas of caseation.

The spleen was much enlarged, measuring 19 by 13 by 9 cm.; it was hard and contained a great number of nodules similar to those in the liver but without the gray peripheral zone. The greatest nodule was 42 mm. in diameter.

The kidney showed sparse dissemination of gray miliary tubercles. The stomach and the small intestine were normal. In the cecum and the ascending and transverse colon there were some circular ulcers, the largest 5 cm. in length, with irregular floor and walls. There were no tubercles in the base of the ulcers or on the peritoneum.

The anterior longitudinal ligaments were separated from the spinal column on both sides of the fifth dorsal vertebra by thin yellow pus. Caries of the fifth and eleventh dorsal vertebrae and of the fifth and tenth intervertebral disks was found. There was an epidural abscess at the level of the eleventh vertebra, without compression of the cord. There was slight angular kyphosis corresponding to the eleventh vertebra. The brain and spinal cord were macroscopically normal.

Microscopic Examination.—Specimens for histologic observation were taken from the skin of the face, epiglottis, larynx, trachea, liver, spleen, periportal lymph nodes, lungs, intestine, kidney and some peripheral (macroscopically normal) nerves, fixed in a solution of formaldehyde and stained with the hematoxylin-eosin, van Gieson, sudan III and Ziehl-Neelsen stains.

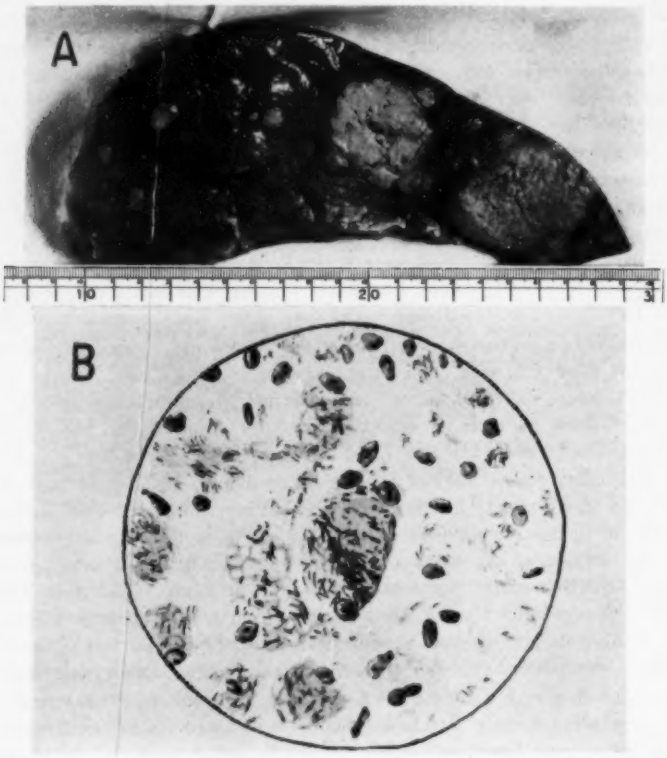
The skin surface was marked by pits up to 3 mm. deep, some with a narrow entrance. In these pits the epidermis was rather thickened and parakeratotic. There was no melanotic pigment in the basal cells in the pits, or in those parts of the free surface where lepra cells were lying immediately beneath the basal cells. In the other parts pigment was found in great quantities. Under the epidermis but separated from it by a layer of fibrous tissue, which was missing only in small zones, there was a wide area of leprous granulation tissue, which will not be described here, as it corresponded to the classic descriptions (for instance, Herxheimer's [2]). Sporadically, giant cells of Langhans and of bone marrow type were found. Virchow's foamy cells were found, with the typically huge number of acid-fast bacilli (fig., *B*) and in sudan stain the lipids stained an orange shade, easily distinguished from the red shade of neutral fat.

In the submucosa of the epiglottis, the larynx and especially the trachea there was a dense infiltration by leprous tissue. The infiltrations were separated from both the epithelium and the deeper layers by fibrous tissue, which sent fiber bundles in various directions into the interior of the leprous tissue. Only beneath the epithelium of the trachea was there no fibrous tissue, and the leprous tissue occurred right under the basal membrane. The cartilage of the epiglottis showed widespread dissolution of ground substance.

In the liver there was congestion with the dilatation of the central veins and capillaries, and great atrophy of liver cells. Throughout the hepatic parenchyma there was dense dissemination of foci, about 0.3 mm. in diameter, of foamy cells,

2. Herxheimer, G.: Virchows Arch. f. path. Anat. **245**:403, 1923.

with wide capillaries; among the foamy cells were found sometimes single atrophic liver cells and small bile ducts. These foci were encapsulated by fibrous tissue, which sent some bundles into the interior of the foci. There were huge masses of acid-fast bacilli in these foci. In other parts there were vast zones of caseation surrounded by tuberculous granulation tissue and fibrous tissue; the former showed lymphocytes, epithelioid cells and a great number of Langhans' giant cells; the fibrous tissue formed a thick layer with bile duct regeneration as in Laennec's cirrhosis; this layer penetrated irregularly between the liver cells. In these fibrotic margins of tuberculous caseation some small groups of foamy cells



A, section through the liver. Two large and several smaller tuberculous masses are seen. *B*, Virchow's lepra cells with vacuoles and bacilli. Ziehl-Neelsen stain; drawing from the original; × 1,000.

were found too. Acid-fast bacilli were seen in great number only in the foamy cells, while in the rest of the marginal zones and inside of the areas of caseation there were acid-fast bacilli only in small number, mostly inside of Langhans' cells.

In a periportal lymph node groups of foamy cells and large tuberculous zones of caseation were found, similar to those in the liver, but there was no development of fibrous tissue around the tuberculous or the leprous foci.

In the spleen was found the greatest number of foamy cells, not in groups, but diffuse in the pulp. There were large zones of caseation, surrounded by tuberculous granulation tissue. No fibrous tissue had developed around these foci.

In the lungs purulent bronchitis and bronchopneumonia were present. There were some groups of foamy cells similar to those in the liver but without the fibrous capsule. There were many typical miliary tubercles with or without central caseation.

In the kidney were some miliary tubercles, composed only of lymphocytes and epithelioid cells. There were only single foamy cells. Parenchymatous degeneration was present.

In an intestinal ulcer there was necrosis of the base down to the limit between the submucosa and the muscularis. The base and the margin of the ulcer did not contain epithelioid or giant cells but contained some lymphocytes and foamy cells. Many foamy cells, although not in groups as in other organs, were seen in the layers immediately above and beneath the muscularis mucosae, in the margin of the ulcer and also in other parts of the colon, which macroscopically were normal. The foamy cells contained acid-fast bacilli in great numbers.

Three peripheral nerves did not show pathologic changes.

COMMENT

The case shows the typical localization of nodular leprosy in the face and in the respiratory tract as well as in most of the inner organs. May I direct attention to the leprous ulcerative colitis, which seems to be the most frequent cause of death in patients with leprosy (Nuñez Andrade [3] and others)? On the other hand, the tuberculosis is marked by extraordinarily large tuberculous foci in liver and spleen. Orth [4] described a tuberculoma, 7 cm. in diameter, in the liver and Fischer [5] another; a few others cited by Gruber [6] and Lubarsch.[7] Gruber [6] expressed the opinion that formation of the large tuberculoma and huge enlargement of lymph nodes may occur frequently in primary infections of adults in districts where tuberculosis is not disseminated. This kind of infection is frequent in Bolivia, for only in recent years has tuberculosis spread in the Indian race. I have found several cases of tuberculoma of the liver, spleen and myocardium, although not of the size now described, in autopsies during the last year. Unfortunately, the kind of tubercle bacillus has not been determined. Whether this form of tuberculosis is provoked only by such circumstances as were noted in the present case, or whether the correlation between leprosy and tuberculosis is also of importance, cannot be decided now, but the latter is not impossible.

In differential diagnosis of tuberculoma of the liver or of the spleen there have to be considered malignant tumor and gumma and in regard to the spleen, also Hodgkin's disease. Leproma of an inner organ has never been found larger than a pea. In the present case the differential diagnosis between leprosy and tuberculosis could easily be established

3. Nuñez Andrade, R.: Medicina, México **19**:173, 1939; abstracted, Bol. Ofic. sanit. Panam. **18**:980, 1939.

4. Orth, J.: Virchows Arch. f. path. Anat. **66**:113, 1875.

5. Fischer, W.: Virchows Arch. f. path. Anat. **188**:21, 1907.

6. Gruber, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 1.

7. Lubarsch, O., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1927, vol. 1, pt. 2.

microscopically by Virchow's foamy cells with an enormous number of acid-fast bacilli, on the one hand, and epithelioid and Langhans' giant cells, caseation and few acid-fast bacilli, on the other. In searching for lepra cells in various organs in routine work sudan III has been the most useful stain because of the clear orange tinge assumed by the lipids in the foamy cells and because of the quickness and reliability of the stain, which may be recommended rather than the Ziehl-Neelsen method, as lepra bacilli do not stain quite reliably by the latter method.

SUMMARY

A case of nodular leprosy is described with typical lesions in the skin of the face, the respiratory tract, liver, spleen, lymph nodes and intestine (ulcers) and, in addition to leprosy, widespread tuberculosis, with miliary tubercles in the lungs and kidneys, as well as caries of two vertebrae. There was also an unusual number of exceptionally large tuberculous masses (tuberculoma) in liver and spleen.

PERSISTENT COMMON ATRIOVENTRICULAR OSTIUM IN A CHILD WITH MONGOLISM

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The high incidence of congenital cardiac defects in persons with mongolism has been noted by several authors (Gibson and Clifton [1]; Morgan and Sprenkel [2]; Leech [3]; Abbott [4]; Lightner [5]; Meeker [6]). Gibson and Clifton [1] stated that 1 out of 6 persons with mongolism has congenital heart disease. One defect in particular, that of persistent atrioventricular ostium with septal defects, has been described frequently enough to be known as the congenital cardiopathy usually found in persons with mongolism. In 1927 Gunn and Dieckmann [7] reviewed the literature and found 22 cases of this defect. They also reported 2 of their own. Of the cases reviewed, however, 16 had been merely cited by Keith,[8] not described, and in all it was said the anomaly was associated with other major defects. Robson [9] cited a case of Mönckeberg and reported 1 of his own; both patients showed mongolism. In 1935 Meeker [6] added another case, thus bringing the total reported to 27. Leech,[3] after analyzing 75 cases of congenital heart disease at Johns Hopkins, reported 3 cases, 2 of them with concomitant mongolism. Morgan and Sprenkel [2] added 1; Ash, Wolman and Bramer [10] found 2 more in a study of 32 patients with congenital heart disease at Children's Hospital in Philadelphia. Gibson and Clifton [1] reported 5 cases of interventricular and interauricular defects occurring together but did not describe the hearts. Goetsch [11] and Lightner [5] added 1 more case. The total of cases reported to date is 39. Of this number, approximately 15 are uncomplicated by other major defects. Over half of the 39 patients had mongolism.

REPORT OF A CASE

A 6 month old white boy was admitted to the hospital Aug. 2, 1940 with cold, fever, cough, hoarseness, anorexia, mild diarrhea and irritability that had been increasing for four days. The child had been born after eight months' gestation, had

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1. Gibson, S., and Clifton, W. M.: Am. J. Dis. Child. **55**:761, 1938.

2. Morgan, D. R., and Sprenkel, V.: J. Tech. Methods **16**:68, 1936.

3. Leech, C. B.: J. Pediat. **7**:802, 1935.

4. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936, p. 50.

5. Lightner, C. M.: J. Tech. Methods **19**:148, 1939.

6. Meeker, L. H.: J. Tech. Methods **14**:72, 1935.

7. Gunn, F. D., and Dieckmann, J. M.: Am. J. Path. **3**:595, 1927.

8. Keith, A.: Lancet **2**:359, 1909.

9. Robson, G. M.: Am. J. Path. **1**:229, 1931.

10. Ash, R.; Wolman, I. J., and Bramer, R. S.: Am. J. Dis. Child. **58**:8, 1939.

11. Goetsch, C.: J. Tech. Methods **18**:117, 1938.

weighed 6 pounds 4 ounces (2,835 Gm.) at birth and had been delivered normally. He had never, however, developed normally and had not sat up at six months. When 6 weeks old, he had had several "blue spells." In April, a roentgenogram of the chest had revealed a large heart; for a few days in May, a discharging ear had been noticed.

The boy was well developed and well nourished but possessed a definitely mongoloid appearance, with almond eyes, smooth nasolabial folds, a broad tongue and stubby hands, extending only to the crests of the ilia. The head was quite brachycephalic. There were an acutely inflamed pharynx, coarse rales over both sides of the upper part of the chest, crepitant rales over the posterior part of the lower lobe of the left lung, an enlarged heart with poorly defined borders, but no cardiac murmurs or abnormal pulsation, a heart rate of 200, a liver margin 4 cm. and a spleen 2 cm. below the costal margin. Examination of the blood gave normal results except for a white blood cell count of 13,100.

While feeding, the patient stopped breathing and became somewhat cyanotic. Oxygen was administered by nose, and aspiration of pharyngeal accumulations was carried out. The patient showed improvement for three hours. Then he suddenly became cyanotic again, had slight convulsive twitchings and died in a few minutes.

The autopsy revealed: congenital heart disease with interatrial septal defect; interventricular septal defect; patent foramen ovale; cardiac hypertrophy and dilatation; pericardial and pleural effusions; pulmonary congestion; fatty change in the liver; hyperemia of the spleen; mongoloid habitus; Meckel's diverticulum.

DESCRIPTION OF THE HEART

The heart weighed 80 Gm. and was globular in shape, showing considerable dilatation of the right auricle and ventricle and moderate dilatation of the left ventricle, but a relatively small left atrium. The chambers on the right side comprised two thirds of the heart. Both ventricular walls were of equal thickness, each measuring 6 mm. The right atrial wall was somewhat thicker than the thin left wall. The pericardium, particularly over the upper portion of the right ventricle, showed numerous small dark red petechiae. Almost no subpericardial fat was present, the coronary vessels being prominently visible. The myocardium was of an even beefy red color, and the mural and the valvular endocardium were smooth and semitranslucent. Postmortem clot filled the dilated chambers.

After removal of much clotted blood from the greatly dilated right atrium, this chamber was half the size seen on opening the pericardial sac. The auricle was greatly dilated and had prominent thin columnae carneae. The upper three fourths of the interatrial septum was present with a sharply outlined limbus fossae ovalis, in the lower portion of which were two small oval defects measuring 4 by 2 mm. each. The ostium of the coronary sinus was wide open but bore a thin rudimentary valvelike fold of endocardium, 1 mm. wide, along its inferior margin. The lower one quarter of the interatrial septum was absent; instead a large round defect was present in communication with a large defect of the upper portion of the interventricular septum, and this common defect directly connected all four chambers of the heart.

This common ostium measured 14 mm. in the vertical axis and 13 mm. anteroposteriorly. The upper margin of the ostium was bounded by a fibromuscular band of the interatrial septum; its anterior and posterior portions were joined at the base

of the auricles. The lower margin was bounded by the round smooth crescentic border of the interventricular septum.

Partially filling the lower portion of the ostium were two common valves, an anterior and a posterior, which ran between the ventricles and functioned as the mitral and the tricuspid valves. Half of each valve lay in the right ventricle, and half in the left. The right half of the anterior valve represented the septal portion of the tricuspid valve, and the left, the aortic segment of the mitral valve. Of the posterior common valve, the right half represented a part of the septal segment and a part of the posterior segment of the tricuspid, while the left half took the place of a part of the posterior leaflet of the mitral valve. Two additional valves

Fig. 1.—The right ventricular wall has been incised close to the interventricular septum and the anterior portion of the right ventricle raised upward, exposing the interior of the right atrium and of the right ventricle. *D.F.O.* indicates defects in the interauricular septum or patent foramen ovale; *O.A.V.*, persistent common atrioventricular ostium; *C.A.V.*, anterior common interventricular valve; *C.P.V.*, posterior common interventricular valve; *A.T.V.*, accessory and rudimentary tricuspid valve leaflets.

were present in the right ventricle to complete the valve ring of the tricuspid; these two were on the anterior wall and took the place of the normal anterior segment. Both were imperfectly formed. The free margins were curled over. The posterior of the two was doubly cleft and thus divided into three equal portions, the free margin presenting a scalloped appearance. The valve leaflets could easily be closed together, and the valve gave the appearance of having been fairly com-

petent. In the left ventricle there was an additional leaflet between the two common valves previously described, the third component of the mitral valve. This segment originated from the superolateral aspect of the left ventricular wall and was attached to both anterior and posterior papillary muscles by long thin chordae tendineae. The valvular orifice was easily closed and appeared to have been competent.

The anterior common valve originated from the upper anterior margin of the common atrioventricular ostium and from the adjacent portions of the right and left atria. It was attached to the right anterior papillary muscle of the right ventricle, to the left anterior papillary muscle of the right ventricle, to the left anterior papillary muscle of the left ventricle and to the lower free margin

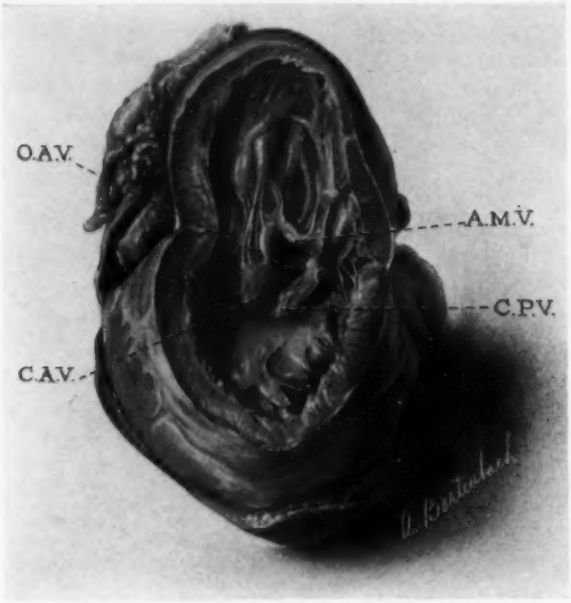

Fig. 2.—The left ventricular wall has been incised close to the interventricular septum and the free portion raised upward, exposing the interior of the left ventricle. *O.A.V.* indicates the persistent common atrioventricular ostium; *C.A.V.*, anterior common interventricular valve; *C.P.V.*, posterior common interventricular valve; *A.M.V.*, accessory mitral valve leaflet.

of the common ostium by numerous thin chordae tendineae. The posterior common valve had a similar attachment, its base being the upper posterior margin of the common ostium. In the left ventricle it was attached by long thin chordae tendineae in the usual manner to the normally placed posterior papillary muscle. The right ventricular attachment, however, was direct to the muscle wall by five double strands of chordae tendineae. Here, also, there were multiple aberrant short chordae tendineae attaching the valve to the posterior free margin of the common ostium. The large common atrioventricular orifice could have been described separately as an interatrial defect and an interventricular defect

separated by the two common atrioventricular valves, but since on separating the free margins of these valves, a common ostium was easily seen, the orifice was considered as one.

The conus was somewhat dilated, and the surrounding ventricular wall was thicker than the rest of the right ventricle. The pulmonary valve consisted of three normally formed cusps, and the orifice measured 2.5 cm. in circumference. The aortic orifice measured 2.2 cm. in circumference and had three normal valvular cusps. Neither the pulmonary artery nor the aorta presented any abnormalities, although the pulmonary artery was visibly slightly larger than the aorta, measuring 3.5 cm. in circumference to 3 cm. of the aorta. The ductus arteriosus was completely closed; it was only a short fibrous cord with a dimple-like depression that could be seen in the intima of each great artery. The great veins entered the heart in the usual manner and presented no abnormalities.

A comprehensive explanation of this developmental anomaly has been given by Mall,[12] Gunn and Dieckmann [7] and Robson.[9] In brief, it may be stated that this defect is the result of an arrest of intrauterine growth, probably in the fifth or sixth week. The ostium primum remains patent because of a failure of the endocardial cushions to grow upward and unite with the lower border of the interauricular septum.

SUMMARY

A survey of the literature finds 39 cases of persistent atrioventricular ostium. Approximately one-half of the patients were mongolian idiots. Over 15 cases were uncomplicated by other major defects. Another case of persistent common atrioventricular ostium in a child with mongolism without other major defects is reported, and the heart described.

12. Mall, F. P.: Am. J. Anat. **13**:129, 1912.

General Reviews

CONSTITUTION AND RELATED FACTORS IN RESISTANCE TO TUBERCULOSIS

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Resistance to invasion by the tubercle bacillus depends on native and acquired factors. The former are the expression of inborn constitution. The latter are brought into operation primarily by environment, but the intensity of their development is a function of innate constitution.

The fundamental characters concerned in the formation of constitution are hereditary. There is abundant evidence that hereditary constitution is important in resistance to tuberculosis. Constitution, however, in the sense now generally accepted, is not solely a product of heredity but is itself in part a product of environment. In the formation of constitution such hereditary factors as racial stock, body build, sex and internal secretion are primarily concerned, but nutrition and other factors, such as the effect of disease, also modify constitution, and often in ways not greatly different from those through which heredity operates. In the following pages constitution, under the broad definition indicated, will be considered in relation to resistance to tuberculosis. It will be convenient to discuss the subject under the following headings:

1. General aspects.
2. Physical habitus and biologic type.
3. Heredity.
4. Race.
5. Age.
6. Sex.
7. Internal secretion and metabolism.
8. Nutrition.
9. Coexisting diseases.

GENERAL ASPECTS

Since early times hereditary constitution and environment have been studied in their relation to the development of tuberculosis. Prior to the understanding of infection constitution was considered paramount,

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although a modifying influence of climate, season and similar factors was recognized. After the discovery that tuberculosis was transmissible, infection obviously became the first consideration, but infection alone was not believed to determine the issue, for all available evidence indicated that other factors remained of importance. At the period of history when the tubercle bacillus was discovered, tuberculous infection at some period of life was nearly universal; yet serious tuberculous disease developed in only a small proportion of the population. Hence for several decades after Koch's discovery in 1882 constitution remained almost as important in general consideration of resistance to tuberculosis as in earlier years when it was believed all important.

In popular conception tuberculosis was associated with a special body type. Hippocrates [1] had written, "The form of the body particularly subject to phthisical complaints was the smooth, the whitish, that resembling the lentil, the reddish, the blue-eyed, the leucophlegmatic, and that with the scapulae having the appearance of wings." Later writers went to greater lengths in describing a "phthisical habitus," and when it became necessary to make this consonant with the concept of infection, it was argued that certain body types afforded a suitable soil or "milieu" for the growth of the tubercle bacillus. In the "Principles and Practice of Medicine," which formed the basis of clinical medicine for a generation of English-speaking physicians, Osler [2] wrote,

> Many years ago I drew the parallel between infection in tuberculosis and the parable of the sower which . . . illustrates in an effective way the importance of the nature of the ground on which the seeds fall. "Some seeds fell by the wayside and the fowls of the air came and devoured them up." These are the bacilli scattered outside the body, an immense majority of which die. "Some fell upon stony places." These are the bacilli that find lodgment in many of us, perhaps with the production of a small focus, but nothing comes of it. They wither away because "they have no root." "Some fell among thorns and the thorns sprang up and choked them." This represents the cases of tuberculosis, latent or active, in which the seed finds the soil suitable and grows, but the conditions are not favorable, as the thorns representing the protective forces of the body get the better of the struggle. "But others fell on good ground and sprang up and bare fruit a hundred fold." Of this fourth group were the 54,435 who died of the disease in 1909 in England—the soil favorable, the protecting force feeble.

The variations in the "soil" making for resistance or susceptibility have been the subject of a large literature. For the early works the

1. Hippocrates: Epidemics, in the Genuine Works of Hippocrates, translated by Francis Adams, Baltimore, Williams & Wilkins Company, 1939, book 3, sect. 3, p. 14.

2. Osler, W.: The Principles and Practice of Medicine, ed. 8, New York, D. Appleton and Company, 1919.

reader is referred to Bauer's [3] monograph on the relation of constitution to internal disease, and for the literature of a somewhat later period, dealing particularly with hereditary aspects of tuberculosis, to Diehl.[4]

Writers of recent texts on tuberculosis (Pottenger [5]; Kayne, Pagel and O'Shaughnessy [6]) have stressed the complexity of interaction between environment, constitution and accident in the final balance, favorable or unfavorable, between infection and resistance. Most writers are agreed on this mutual interaction, and some consider it too complicated to unravel. Others have urged research in definite directions. Miller,[7] emphasizing the disparity in numbers between the many infected and the relatively few seriously diseased, wrote "the study of resistance is of as great if not greater importance than the study of infection. . . . Natural or constitutional resistance would appear to be a real thing, as evidenced by the great variation in susceptibility in individuals, families and races." Ryder,[8] calling the tubercle bacillus a "universal virus," drew attention to the broad difference in the effects of tuberculosis and certain other infectious diseases also infecting all the population but conferring a definite immunity; in the latter case acquired immunity determined the issue, as well as the response to subsequent attacks, whereas in the case of tuberculosis survival appeared to depend on innate resistance. Kayne, Pagel and O'Shaughnessy [6] considered inborn resistance "of far greater significance than the resistance acquired as the result of past infection."

The most recent tendency is to emphasize the modifications that environment itself can effect in constitution. As Barker [9] wrote, physicians cannot change the genotypic constitution, but they can alter the "paratypic" constitution. As he stated, "constitution is not a fixed, unalterable entity." The role of diet and nutrition in modifying constitution has been emphasized by Heise [10] in a recent review of the whole subject of constitution and environment in tuberculosis.

In the opinion of a few writers hereditary constitution is so important that the outcome of infection is determined almost solely by its relation

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3. Bauer, J.: Die konstitutionelle Disposition zu inneren Krankheiten, Berlin, Julius Springer, 1924.

4. Diehl, K.: Ergebn. d. ges. Tuberk.-forsch. **3**:137, 1931.

5. Pottenger, F. M.: Tuberculosis in the Child and the Adult, St. Louis, C. V. Mosby Company, 1934.

6. Kayne, G. G.; Pagel, W., and O'Shaughnessy, L.: Pulmonary Tuberculosis: Pathology, Diagnosis, Management and Prevention, New York, Oxford University Press, 1939.

7. Miller, J. A.: J. A. M. A. **111**:111, 1938.

8. Ryder, C. T.: Am. Rev. Tuberc. **17**:103, 1928.

9. Barker, L. F.: Am. Rev. Tuberc. **30**:519, 1934.

10. Heise, F. H.: Am. Rev. Tuberc. **43**:245, 1941.

to primary disposition and age. In discussing the age periods of most risk from tuberculosis Wallgren [11] wrote:

> The first prerequisite that a pulmonary tuberculosis shall develop is a tuberculous infection. . . . Only a small part of all infected develop lung tuberculosis. . . . The second prerequisite is a disposition, and among all the constitutional and conditional factors which together constitute the concept of disposition to consumption, the most significant are heredity and that the age of puberty shall have been reached. . . . In a small part of all infected persons . . . there is a disposition to lung tuberculosis, and of these infected individuals the majority develop consumption.

This is an extreme view, but quite as strong pronouncements have been made on the determinative influence of environment.

PHYSICAL HABITUS AND BIOLOGIC TYPE

Physical Habitus.—Certain body types have long been associated in both popular and medical conception with pulmonary tuberculosis. It has been generally recognized also that cause and effect must both be considered in explaining the association. The shape of the thorax has been most stressed. It is generally agreed that chronic pulmonary tuberculosis ultimately affects the appearance of the thorax. Certain writers, however, have claimed that the primary shape determines to some extent whether tuberculosis will occur or not. Draper,[12] a leading investigator in the latter group, in recording his own experience, wrote, "The thorax has been the object of more anthropometric study by the clinician than any other part of the body." He concluded from long study that the thorax of the tuberculous subject is flat and narrow and that in spite of its narrowness it is relatively more flat in respect to both width and length than other types. He found it relatively longer than wide, with a small subcostal angle and a short circumference.

This is the traditional view, but it has been contested. Recently Weisman [13] published experience leading to the conclusion that the round chest represented a predisposition to tuberculosis. His study was based on the examination of several hundred normal subjects and tuberculous patients at the University of Minnesota and several neighboring sanatoriums. His results indicated that the ratio of anteroposterior to transverse chest diameter in normal subjects of early adult age ranged from 0.67 in men to 0.70 in women, while in the tuberculous patients measured, who averaged 37 years of age, the range was from 0.726 to 0.773.

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11. Wallgren, A.: Acta tuberc. Scandinav. **10**:321, 1936.

12. Draper, G.: Human Constitution: A Consideration of Its Relationship with Disease, Philadelphia, W. B. Saunders Company, 1924.

13. Weisman, S. A.: Your Chest Should Be Flat, Philadelphia, J. B. Lippincott Company, 1928.

A much more complicated picture of the relation of body build to tuberculosis has been built in continental Europe in the years since Stiller [14] developed the conception of the asthenic habitus (1907). For the early literature the reader is referred to Bauer.[3] More recently several anthropologic classifications have been made. Ickert [15] has published his experience based on separation of patients according to the Kretschmer [16] classification, in which persons are classified as asthenic or leptosomic, pyknic and athletic. The asthenic ones are slender, long chested and delicate, the pyknic ones stocky, and the athletic ones well developed and muscular. In Ickert's experience tuberculosis rarely developed in the athletic type. The majority of tuberculous patients in his experience were of the leptosomic type. In intermediate types the relations were complex. No reason could be advanced for the apparent greater susceptibility of the leptosomes; it was assumed that the body type itself was the result of certain more fundamental characters, including the action of the vegetative nervous system and the organs of internal secretion, which might affect tuberculosis in ways independent of body build.

Results of a different nature were reported by F. Potthoff,[17] who made careful anthropologic studies of a thousand tuberculous adults. He was unable to detect a particular shape of thorax among them, and indeed found that the chest measurements of tuberculous patients corresponded well with those of the corresponding general population. Contrary to the usual report, he found no preponderance of the asthenic type of body contour in tuberculous patients. He concluded there was no measurable constitutional structure characteristic of tuberculosis or indicating a disposition to it.

A study by A. Potthoff [18] of tuberculous children, on the other hand, seemed to indicate some relation between tuberculosis and body build. No particular relation was apparent as far as the primary and secondary phases of the disease were concerned, but a high proportion of children in the so-called tertiary phase of tuberculosis were leptosomes. Leptosomic character seemed commonly associated with an exudative nature of the tuberculosis. Pryor and Mathiasen [19] had previously reported somewhat similar conclusions in comparing 210 tuberculous and 6,000 healthy children. A consistent trend toward the slender build was noted in the tuberculous children.

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14. Stiller, B.: Die asthenische Konstitutionskrankheit, Stuttgart, Ferdinand Enke, 1907.

15. Ickert, F.: Beitr. z. Klin. d. Tuberk. **72**:774, 1929.

16. Kretschmer, E.: Körperbau und Character, ed. 3, Berlin, Julius Springer, 1922.

17. Potthoff, F.: Beitr. z. Klin. d. Tuberk. **89**:180, 1937.

18. Potthoff, A.: Beitr. z. Klin. d. Tuberk. **92**:612, 1939.

19. Pryor, H. B., and Mathiasen, H.: Am. Rev. Tuberc. **33**:348, 1936.

In considering body build mention should be made of the special susceptibility of the pulmonary apex, which has been attributed to various mechanical factors supposed to lower its resistance (Kayne, Pagel and O'Shaughnessy [6]). It has been claimed that in asthenic persons visceroptosis draws down the diaphragm with a vertical pull on the pulmonary apex, dilating the lung tissue and thereby impairing its resistance. Other investigators have claimed the apex is good soil because of its restricted motion, while still others find in the restricted motion the explanation of the resistance in the apex. However, it has never been proved to the satisfaction of physiologists that one part of the lung functions differently from another. The reader is referred to the lengthy review by Loeschcke and Dehoff [20] on the disposition of the pulmonary apex to tuberculosis.

Of somewhat similar import is the possible predisposition to tuberculosis resulting from fixation of the upper part of the lung by healed tuberculosis at the root. Macklin [21] suggested that restriction of root motion might predispose to tuberculosis through an adverse effect on movement of the lung as a whole.

Studies on body build have dealt chiefly with the skeletal shape. Much less frequently the relation between tuberculosis and the body covering has been investigated. A clinical impression exists that fat persons who acquire tuberculosis do not do well. The problem is complicated by the fact that long before tuberculosis is recognized it may have led to considerable loss of weight. In a review of studies on 2,701 patients in a clinic of Rostock, Germany, Curschmann [22] reported that only 41, or 1.51 per cent, were fat, a number considerably below that in the general population. The course of disease in the majority of these was unfavorable.

Traditionally, blondness has been associated with predisposition to tuberculosis (Hippocrates [1]), although recently in Germany, whence most of the literature on the subject has emanated, blond complexion has come to connote a favorable prognosis. Blondness is variable, and intermediate varieties make statistical investigation difficult. A study that seems conclusive as far as the type with red hair is concerned was made by Bogen,[23] based on the sanatorium admission records of 10,000 patients. The course of minimal, moderately advanced and far advanced pulmonary tuberculosis and childhood and nonpulmonary forms appeared to be no different in red-haired patients than in other white patients, and a few examples of unusually high resistance were found in those with red hair.

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20. Loeschcke, H., and Dehoff, E.: Ergebn. d. ges. Tuberk.-forsch. **2**:81, 1931.
21. Macklin, C. C.: Am. Rev. Tuberc. **25**:393, 1932.
22. Curschmann, H.: Beitr. z. Klin. d. Tuberk. **89**:556, 1937.
23. Bogen, E.: Am. Rev. Tuberc. **43**:285, 1941.

Biologic Type.—In Germany a type of constitution with prominent development of lymphoid tissue and ready response to inflammatory stimuli has been recognized as the "exudative or exudative-lymphatic diathesis." This type is considered resistant to tuberculosis (see Bartel [24] for early literature). According to Klare,[25] this type is much superior to the "asthenic" type of Stiller in the readiness with which it responds to injury by production of antibodies and new growth of tissue. Klare and Böhning [26] have written a monograph on the question of constitution and tuberculosis as elucidated in a study of the course of the disease in 190 children, nearly equally divided among those with favorable and those with unfavorable course. Out of this and other observations came a concept of constitutional factors in relation to tuberculosis, which Klare [27] has put together in a simple schema for clinical use. According to his formulation, the studies on physical habitus have not been definitive, while relations with physiologic and functional behavior can be noted unmistakably. Factors to be considered as of favorable import in the event of tuberculous infection were listed by Klare as follows: occurrence before puberty, absence of tuberculosis in the family, light eyes, blond hair, tender red skin, large tonsils and cervical lymph nodes, a pharynx rich in lymphoid tissue and, finally, dermographism. The opposites of these, including dark, hard skin, poor development of lymphoid tissue and weak vascular response in the skin, indicated a relatively poor prognosis.

Debate has taken place between Klare and Kutschera-Aichbergen [28] on the relation of extraneous factors to constitution. The latter agreed with Klare that the physiologic is of more significance than the anatomic makeup in resistance to tuberculosis but laid more emphasis than Klare on heredity and the modifying influence of environmental experience.

An extensive study of physiologic indexes in tuberculous and nontuberculous patients was made by Petersen and Levinson.[29] In a group of supposedly normal men 9 patients with active tuberculosis were discovered. These showed a low inflammatory index (based on the relation of blister time and capillary permeability, as determined by appropriate methods), slow reaction to ice and increases in serum globulin, basal metabolic rate, muscular irritability and pulse rate. Some of these characteristics were considered the result of tuberculosis. Others were

24. Bartel, J.: Zentralbl. f. d. ges. Tuberk.-forsch. **17**:389, 1922.

25. Klare, K.: Deutsche med. Wchnschr. **64**:845 and 887, 1938.

26. Klare, K., and Böhning, F.: Die offene Lungen-Tuberkulose bei Kindern und Jugendlichen. Ein Beitrag zur Frage: Tuberkulose und Konstitution, Leipzig, Georg Thieme, 1938.

27. Klare, K.: Ztschr. f. Tuberk. **80**:65, 1938.

28. Kutschera-Aichbergen, H.: Wien. klin. Wchnschr. **51**:893, 1938.

29. Petersen, W. F., and Levinson, S. A.: Arch. Path. **9**:147, 1930.

thought to be inherent characters and of prognostic significance. This group of men, with no symptoms of toxemia, were considered resistant. A further study of 83 tuberculous patients in a sanatorium correlated low permeability of capillaries and a low inflammatory index with good prognosis and indicated that in general patients with strong sympathetic-cotonic reactions did well. Patients in whom the course of the disease was favorable gave relatively strong reactions to tuberculin. Aside from this, however, low reactivity of the skin seemed an advantage.

It has been claimed that the teeth are good indicators of the prognosis of tuberculosis. According to King-Turner,[30] active caries of the teeth is associated with active tuberculosis. He thought that a similar mechanism might underly both conditions, viz., an abnormal blood reaction leading to insufficient ionic calcium to calcify lesions in the lungs. It has never been proved, however, that the calcifying process per se cures tuberculosis.

Human Response to Artificial Inoculation of Tubercle Bacilli.—In all the articles cited thus far a weakness in argument on the influence of constitution is evident in the fact that the dosage of bacilli was quite unknown. Unless the dosage is known there is no proof of supposed constitutional differences. It has been hoped, therefore, that some information on constitution will come from the studies on inoculation of human subjects with living bacilli for prophylactic purposes. The Lübeck disaster, in which 251 newborn children were inoculated with living virulent tubercle bacilli, substituted by mistake for BCG, seemed to offer some return in this respect for the tragedy of the event.[31] Superficially there is evidence for a marked innate difference in the children. All were inoculated in the first ten days of life. Seventy-six, or 30 per cent, died of massive tuberculosis in the first year. One died in the second year, and since then there has apparently been no significant mortality. The surviving children have all been subjected to continuing clinical study, and all evidence, including that from roentgen examination of the abdominal lymph nodes, indicates that they have recovered from extensive primary tuberculosis. At first sight, the dosage and method of administration having supposedly been the same in all children, the early death of one group and the continuing survival of another seems evidence of special innate resistance in the latter. However, closer examination indicates that this conclusion cannot be drawn. There were many inconstant factors in the inoculation, including the manner of preparation of the inoculum, the age of the culture employed, the amount of suspending fluid used and the temperature of the fluid. In addition,

30. King-Turner, G. E.: Tubercle **20**:311, 1939.

31. Die Säuglingtuberkulose in Lübeck, in Arbeiten aus dem Gesundheitsamte, Berlin, Julius Springer, 1935, vol. 69.

there was inconstancy in the presence or absence of food in the stomach and in such factors as vomiting and diarrhea. All of these factors were carefully weighed by various investigators. Certain groups of the children seemed to be comparable, and when these were classified according to apparent manifestations of the "exudative diathesis," some benefit from the latter appeared evident. However, the differences were not great, and, on the whole, conclusive evidence as to the role of constitution is lacking.

Recent studies of response to inoculation with true BCG may have significance. Levine [32a] compared the response of 74 white and 38 Negro infants to intracutaneous inoculation of BCG and found a much greater tendency to severe reaction in the latter. Necrotizing lesions developed at the site of inoculation in 85 per cent of the Negro children and abscesses developed in the draining lymph nodes in 47 per cent, whereas only 44 per cent of the white children showed necrosis at the site of inoculation and 23 per cent lymph node abscesses. Kristenson [32b] made a careful analysis of the local response to BCG vaccination in a group of young men and women in Uppsala, Sweden, who previously had given negative reactions to tuberculin. He classified them according to physical types on the Kretschmer [16] basis. The results indicated that the most rapid and strong inflammatory response occurred in the pyknic subjects and the least vigorous reaction in the leptosomic subjects. The reactions to tuberculin were parallel in their intensity. This type of investigation has great advantages for the study of constitutional factors, and it is to be hoped that other studies of inoculated subjects will be made.

HEREDITY

"Tuberculosis families" have been recognized since early times. Before the infectious nature of tuberculosis was known, the continuing presence of tuberculosis in such families was attributed to hereditary susceptibility. After the contagious character of tuberculosis became apparent, the phenomenon seemed most reasonably explained by the continuing transmission of infection through succeeding generations. However, numerous investigators were not satisfied with this explanation and have made analyses of the significance of heredity, with supposedly full allowance for the infectious element.

Studies of Family Lines.—Among the interesting analyses of family successions are those by Pearl,[33] whose reports may be taken as typical of a large group in this field. Starting on the common assumption that most persons are infected while but few succumb, Pearl traced the

32. (*a*) Levine, M. I.: Am. J. Dis. Child. **51**:1052, 1936. (*b*) Kristenson, A.: Acta tuberc. Scandinav. **14**:1, 1940.

33. Pearl, R.: Am. Rev. Tuberc. **4**:688, 1920; Studies in Human Biology, Baltimore, Williams & Wilkins Company, 1924.

course of clinical and fatal tuberculosis in certain families of supposedly like social and economic environment. His studies showed in general that when the amount of tuberculosis in the direct ancestry was high the amount in the offspring was proportionately great. It was evident that the rate of close contact with persons suffering from open tuberculosis corresponded, and the assumption might have been made that therein lay the entire explanation of the high rate in the offspring. However, virtually three quarters of the nontuberculous offspring were in just as close contact as their less fortunate brothers and sisters. Pearl's [34] later studies confirmed his views on the importance of heredity but indicated great difficulties in any attempt to apply the theories of heredity based on experimental work or to interpret the data obtained from observation of human subjects on simple mendelian schema.

Studies of family lines have been made by a number of investigators in an attempt to find grounds for special susceptibility. Münter [35] reported on the causes of death in a certain rural community in Baden, Germany, for the period from 1852 to 1926, giving histories of 33 families, some of which could furnish clear lines of descent from the seventeenth century. Münter's study led him to conclude that certain families had inherent pulmonary susceptibility, making them subject to pulmonary infections in general. The hereditary disposition appeared to be recessive but played a significant role in the long family history.

A similar approach was made by Berghaus,[36] who followed the course of extrapulmonary tuberculosis as an index of predisposition in 769 family lines with a membership of 16,551 persons, of whom 2,888 sickened or died of tuberculosis. He considered extrapulmonary tuberculosis as more significant for such a study than pulmonary, because of its relative infrequency and the lesser influence of environment in its causation. In many family lines he noted a special concentration of tuberculosis of bones and joints or of tuberculosis of the skin. Occasionally, anomalies of the organ concerned were encountered in other members of the family, and Berghaus was led to think of an inherited locus minoris resistentiae. This in his view was nonspecific, simply favoring seeding by tubercle bacilli. Thus tuberculous predisposition was to be considered a negative quality. A reviewer, Diehl,[37] wrote, "I believe this family line gives the key to the problem of tuberculosis inheritance."

Disappearance of tuberculosis in family trees is often as impressive as the continuous sequence of the disease, and this, too, is believed to have an explanation in terms of heredity. Drolet [38] considered some of

34. Pearl, R.: Zeitschr. f. Rassenk. **3**:301, 1936.

35. Münter, H.: Beitr. z. Klin. d. Tuberk. **76**:257, 1930.

36. Berghaus, W.: Arb. a. d. Staatsinst. f. exper. Therap. **36**:1, 1938.

37. Diehl, K., in abstract of Berghaus,[36] Zentralbl. f. d. ges. Tuberk.-forsch. **50**: 177, 1939.

38. Drolet, G. J.: Am. Rev. Tuberc. **10**:280, 1924.

the factors responsible, writing of an "increased immunity by hereditary evolution." One of the most notable studies of the disappearance of tuberculosis in families has been reported by Geissler,[39] who continued a family analysis commenced by Riffel more than thirty years previously. The family lines studied were traced to a couple married near the end of the eighteenth century, 5 of whose children died of tuberculosis. Up to 1860 about half of the descendants had acquired the disease, and in 1867 the intrafamilial epidemic reached its peak. At the time of Geissler's report, in 1938, the disease in the family lines had become almost extinct. In some branches of the family tuberculosis did not occur after 1900. Geissler considered the factor of environment and exposure but attributed the decline of tuberculosis to outbreeding. From his experience he developed a formula with respect to hereditary resistance; in his view this represented a balance of three factors, rather than the influence of a single hereditary factor, viz., two concerned with susceptibility and one with nonspecific resistance, the latter being under the influence of environment. Physical habitus did not seem of importance; the leptosomic character, in a high degree conditioned by heredity, did not show any characteristic association with tuberculosis in the family line.

In another publication Geissler [40] reviewed the subject of heredity in its various aspects and stated his conclusion that at least a third of the German people are hereditarily resistant to tuberculosis.

Other family studies in Germany have not led to identical conclusions, however. In an extensive study involving 129 infants, 185 preschool children, 7,214 school children and 2,384 adults, Schremf [41] concluded that, although heredity was of significance, environment was of primary importance and the course of first infection, at least, was determined by the massiveness of the dose. The leptosomic type did not seem subject to more severe illness as compared with other types.

The disappearance of tuberculosis or of any infection in a family line need not be attributed solely to the introduction of new stock by cross breeding. Any original population forming the basis of study may exhibit wide variations in resistance, subject to natural selection. That such selection will occur has been shown unequivocally by Webster, who has studied epidemiology experimentally for fifteen years in inbred families of mice. The most important conclusions are presented in nontechnical form in a recent review. Webster [42] found by experiment on a

39. Geissler, O.: Beitr. z. Klin. d. Tuberk. **91**:1, 1938.

40. Geissler, O.: Zur Frage des Erbgangs der Tuberkulose-Fälligkeit, eine Auswertung der Ergebnisse klinischer Konstitutionsforschung, Leipzig, Georg Thieme, 1939.

41. Schremf, K.: Beitr. z. Klin. d. Tuberk. **84**:508, 1934.

42. Webster, L. T.: Scient. Monthly **48**:69, 1939.

large series of mice subjected to similar infection with Bacillus enteritidis that those succumbing early bore progeny more susceptible than did those who escaped serious disease. With this lead, he inbred strains of mice for many generations, selecting for resistance and susceptibility, with the final result of two strains indistinguishable in appearance but differing ten thousand fold in their resistance to the infection with reference to which they were selected. By setting up sample populations with varying proportions of the two types and exposing them to infection, he was able to show that with extinction of the susceptible stock the rate of mortality rapidly declined. Heavy replacement with susceptible mice, for which the rate could be calculated, was required to maintain a constant mortality.

That neither natural selection by survival nor change of environment to one of less exposure is essential in decline of mortality in a population is shown by certain studies of Diehl [43] on the number of children begotten by tuberculous parents at different ages. These studies, carried out on 1,115 patients, indicated that, even if they survived, tuberculous parents begot few children after onset of the disease in comparison with the number they begot before the disease began. The net result of selection by tuberculosis on this basis would be a shortening of the procreative period and a corresponding decline in the descendants of tuberculous ancestors.

Tuberculosis in Twins.—If hereditary factors are of great importance in resistance to tuberculosis, single egg twins should show similar behavior toward tuberculous infection, while two egg twins might or might not behave similarly, according to chance. Diehl and Verschuer [44] made a study, now widely quoted, of 106 pairs of twins in which at least one member was clinically tuberculous or gave evidence of significant infection in roentgen films; 37 pairs were identical (monovular) and 69 nonidentical (binovular) twins. A concordance of behavior in respect to tuberculosis was found in 26, or 70 per cent, of the pairs of identical twins and in only 11, or 30 per cent, of the pairs of nonidentical twins.

The evaluations of response were made in the following categories: (1) both twins sick or dead, (2) both with slight evidence of tuberculosis but clinically well, (3) one clinically well and the other ill or dead and (4) one dead, the other ill. The clinically well group included those with roentgen evidence of pulmonary calcification, fibrous scarring or both. The investigation of the subjects included an elaborate physical examination as well as an intimate study of environment and history.

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43. Diehl, K.: J. A. M. A. **108**:408, 1937.

44. Diehl, K., and Verschuer, O.: Zwillungstuberkulose, Zwillungsforschung und Tuberkulosedisposition, Jena, Gustav Fischer, 1933.

In the monograph by Diehl and Verschuer all of the cases are described at length. With the primary fact of a majority concordance in behavior toward tuberculosis apparent in single egg twins, they investigated possible factors for hereditary susceptibility or resistance to the disease. Three possibilities were considered, viz., a *specific disposition* to tuberculosis, which would become evident in the face of a universal infection, a *nonspecific disposition,* operating through a number of hereditarily conditioned body states, and a characteristic *behavior pattern,* nearly constant for single egg twins and inconstant for two egg twins.

The investigation at its conclusion failed to yield any evidence of tangible factors for resistance or for susceptibility. Elaborate anthropometric studies led to the conclusion that differences in general body build and in structure of the thorax were not concerned. Sex made no appreciable difference. In the group of two egg pairs in which the twins were of different sex, the incidence of tuberculosis was equally divided among males and females. A slight suggestion of influence of sex was found in the fact that tuberculosis developed somewhat more frequently in girls who began to menstruate early than in those in whom the onset occurred at the normal time. There was no association of color of hair or color of eyes with tuberculosis. There was no significant difference in the susceptibility of members of different blood groups. No relation was found between the condition of the thyroid gland, traditionally associated with resistance to tuberculosis, and the onset of disease in the cases studied, but the number of cases in which the thyroid was abnormal was small. No substantial evidence of the role of the "exudative lymphatic diathesis" was obtained, although good resistance was apparent in a small number of persons with excessive development of lymphoid tissue.

In the final analysis Diehl and Verschuer were unable to identify anything of a specific or a nonspecific constitutional nature to which resistance or susceptibility to tuberculosis could be attributed. They concluded that a single or a multiple hereditary "anlage" of admittedly unknown character acted in such manner that the carrier was likely to display great susceptibility to tuberculosis, once infected. Correspondingly, persons without the responsible genotypes would display high resistance.

The investigations have been continued since publication of the well known monograph. According to a recent report, similarity of pulmonary and hilar configuration is apparent in a high percentage of single egg twins.[45] This might well be expected, and thus far Diehl and Verschuer have not reported any progress in the identification of specific factors.

45. Diehl, K.: Beitr. z. Klin. d. Tuberk. **93**:221, 1939.

Other studies of tuberculosis in twins are on record. Diehl and Verschuer have themselves reviewed the earlier ones. Of the later ones, that by Uehlinger and Künsch[46] is most significant. These authors, studying 46 pairs of twins (12 single egg and 34 two egg), came to conclusions similar to those reached by Diehl and Verschuer, although the degree of concordance shown by single egg twins in the character of their response to tuberculosis was not as high as that reported by the latter authors. Uehlinger and Künsch also were unable to identify specific factors and concluded that the similarity in the response of single egg twins could be attributed only to a specific genotypic disposition to tuberculosis.

Other studies, such as those of Berghaus,[47] may be cited, but these are of less importance than those described at length, since no differentiation into single ovum and two ovum groups was made.

Experimental Studies on Heredity in Tuberculosis.—Experimental studies have a greater value than observations on human tuberculosis, because the factor of environment can be controlled. Species differences in animals in response to artificial inoculation are well known. They have been subjected to careful research by Vorwald,[48] who compared resistance and inflammatory response, noting both qualitative and quantitative differences among strains in the latter respect. It appeared that resistance could not be correlated with intensity or character of inflammatory response, and Vorwald concluded that the capacity of the tubercle bacillus to multiply in tissues depended on other factors.

Differences in susceptibility within a single strain of animals have been described in a number of important publications. These differences have a bearing on the problem of human tuberculosis through analogy. In 1921 Wright and Lewis[49] and in 1923 Lewis[50] reported a study of experimental tuberculosis in 5 families of guinea pigs from the stock of the United States Bureau of Animal Industry, with 11 generations of brother and sister mating behind them. The members of these families were considered homogeneous in heredity in their respective groups and possessed contrasting characteristics in size, fertility, and pattern of coat. Representatives of the several families were infected with tubercle bacilli of the human type. Definite differences in familial susceptibility became apparent, especially as indicated by length of survival. The most resistant families survived twice as long as the least resistant. From statistical calculations the authors concluded that 10 per cent of the

46. Uehlinger, E., and Künsch, M.: Beitr. z. Klin. d. Tuberk. **92**:275, 1938.

47. Berghaus, W.: Arb. a. d. Staatsinst. f. exper. Therap. **36**:68, 1938.

48. Vorwald, A. J.: Am. Rev. Tuberc. **27**:270, 1933.

49. Wright, S., and Lewis, P. A.: Am. Naturalist **55**:20, 1921.

50. Lewis, P. A.: The Relation of Heredity to Tuberculosis, in Eugenics, Genetics and the Family, Baltimore, Williams & Wilkins Company, 1923, p. 178.

variation in length of life could be attributed to differences in condition, weight and age but that 30 per cent must be due to heredity. From the nature of the experiment a large amount of accidental variation was to be expected, and 50 to 60 per cent of the variation in survival time was credited to unknown causes.

The families selected for study proved of low, intermediate and high susceptibility. The capacity for resistance appeared to be transmitted by each sex to the offspring of each sex, and this was true of crosses with other inbred families. In crosses among the susceptible families the resistance of the progeny did not exceed that of the parent stock. The factors determining resistance were not identified. Resistance did not appear definitely associated with vigor, growth rate, adult weight, size and frequency of litter, percentage of young born alive or percentage of young raised to the time of weaning. Sex made no appreciable difference in resistance, and age within the period of inoculation also appeared not to be a factor.

Subsequent experiments by Lewis and Loomis [51] resulted in a correlation between resistance and inflammatory response to the infecting bacilli. The experiments were carried out by intracutaneous inoculation. In all cases the immediate reaction was the formation of a papule which became a nodule and then ulcerated. There was great variation among the families, however, in the character of the procession of change. In the family of highest resistance, as determined by survival time after infection, a compact nodule developed, with a relatively restrained type of ulceration. In the family with lowest resistance a soft, poorly demarcated edematous nodule developed, which was succeeded by destructive ulceration. Corresponding skin responses to other irritants than the tubercle bacillus, such as turpentine and cantharides, were shown by these families, and the authors concluded that the hereditary distinctions in resistance among families were related to the general quality of inflammatory reaction.

Küster and Krönig [52] reported a similar experience based on the use of inbred lines of guinea pigs from the Zoological Institute of Göttingen, Sweden. About 2,000 animals in 14 series were infected by a variety of methods with the same strain of tubercle bacilli. Guinea pig lines of high, low and intermediate resistance were encountered. Heredity appeared of influence in survival time, weight lost during illness and characteristic localization of the disease. Specific genotypic characters could not be recognized. Color of coat and length and form of hair did not seem related to resistance. Age appeared of slight influence,

51. Lewis, P. A., and Loomis, D.: J. Exper. Med. **47**:449, 1928.

52. Küster, E., and Krönig, F.: Arb. a. d. Staatsinst. f. exper. Therap. **35**:38, 1938.

in that young animals seemed a little more susceptible, and the offspring of young mothers was less resistant than that of old. While the involvement of certain organs was similar in the different guinea pig lines, there was great variation among these in the amount of splenic infection.

A more exhaustive experimental study of heredity has recently been reported by Lurie.[53] Six rabbit families were inbred by brother and sister or by parent and offspring matings through five generations. Representatives of each generation were infected with bovine type tubercle bacilli and their resistance compared. Various methods of infection were tried, but natural contagion proved the most fruitful. A large cage was used, separated in the center by two layers of coarse and one of fine mesh, which prevented contact and alimentary infection but permitted air-borne infection. Tuberculous rabbits on one side of the cage, shedding tubercle bacilli freely through the urine into the bedding, stirred up infected dust, which passed through the mesh in small amounts to the other side, where the representatives of the families under study were placed. The latter were housed in separate cages, the location of which was changed each day to equalize any environmental differences from chance local concentrations of the rabbits which served as sources of contagion.

With remarkable constancy in successive generations, members of the 6 families displayed resistance making possible their classification in three groups. One family was characterized by slow progression of the disease, long life after infection, encapsulation of lesions, slow ulceration and excavation in the lungs, bronchogenic spread of infection within the lungs, relative freedom from tuberculosis in the tracheobronchial lymph nodes and minimal amount of hematogenous extension. In contrast, two other families throughout the experiment exhibited tuberculosis characterized by a progressive and rapid course, caseopneumonic development without tendency toward encapsulation, sequestration of large masses of tissue rather than slow ulceration, massive caseation of the tracheobronchial lymph nodes and abundant hematogenous dissemination. In the remaining families the tuberculosis was intermediate in course and character. The two extremes, although both apparently the result of progressive primary infection, in anatomic character were analogous to chronic reinfection type and massive primary type tuberculosis in man.

Microscopic examination of tissues from animals succumbing from the various series showed a constant relationship with respect to the cellular reaction to tubercle bacilli. In the resistant family the large mononuclear phagocytes seemed able to inhibit the multiplication of

53. Lurie, M. B.: Am. Rev. Tuberc., to be published.

tubercle bacilli in some degree, whereas this capacity was lacking in the mononuclears of the susceptible animals, which literally swarmed with tubercle bacilli in the infected regions.

Other methods of infection led to the same differentiation as long as the infecting dose of bacilli was kept small. This was especially well shown on infection with known doses by inhalation in a specially devised chamber. Large doses overwhelmed the animals, with rapid fatal issue, regardless of the family of the experimental subject. With small doses, on the order of 25 bacilli or 25 minute air-borne clumps of bacilli, differences of character identical with those shown through natural contagion from exposure to infected rabbits were evident. Parenteral infection with small doses led likewise to the same result.

Thus there seemed no doubt that a true difference between strains existed, and since this bred true in generation after generation, it appeared to be on a hereditary basis. Sex, age and color of coat were not associated with differences in resistance. In other words, the transmissible characters affecting resistance were not sex linked, and resistance could not be predicted from the appearance of the animals.

Lurie and his associates tested a variety of possibilities in search for characteristics that might explain the differences in resistance. The factor of chief importance proved to be a fundamental difference in the behavior of the tissues at the portals of entry in the different animal families. The difference was analogous to that discovered by Lewis and Loomis.[51] Resistance to tuberculosis seemed intimately associated with the intensity and rapidity with which local immunity developed at the point of intracutaneous infection. In the resistant animals the lesions reached a peak of reactivity quickly and subsided rapidly. Almost complete healing resulted as a rule, although late exacerbation occurred in a few instances. In contrast, rabbits from the susceptible families exhibited slow development of the intracutaneous lesion with late and ineffective tendency to heal. Exacerbations of the local lesions were the rule. The animals infected by the intracutaneous route exhibited exactly the same variation in general resistance as was shown by the different families in the inhalation experiments.

In addition to the difference in the cellular reaction, previously mentioned, the two most sharply contrasting families varied from each other in certain other detectable ways. Members of the resistant family showed relatively low cutaneous permeability to dyes and particulate matter like india ink, a rapid and relatively intense development of allergic sensitivity and a high development of agglutinins for heat-killed tubercle bacilli. In other words, the animals with low resistance displayed this character not only in an inherent poor response to infection but in their inability to be effectively immunized. In passing it may be

stated that it has been repeatedly suggested on observational evidence that a similar variation occurs in human stocks.

Lurie's final conclusion was that genetic constitution is responsible for variations in resistance but that this operates through combination of a number of factors.

Tuberculosis and Mental Disease.—A relation not merely dependent on environment has long been suspected for tuberculosis and schizophrenia. The reader is referred to an article by Westphal and Welti [54] for early literature on the subject and to Kallman's [55] monograph for a more recent review. Early studies of families indicated that not only schizophrenic persons themselves but also their nonpsychotic blood relatives had a much higher mortality from tuberculosis than the general population. Kallman made an extensive study of tuberculosis in the families of schizophrenic patients residing in or near Berlin, Germany. The study involved investigation of family and hospital records remote from that city. Of the several possible means of studying the relative susceptibility of schizophrenic and nonschizophrenic families, that chosen was the ratio of the number of schizophrenic persons dying of tuberculosis to the number dying from all causes. A very high percentage of deaths from tuberculosis was found for the children of known schizophrenic persons. The preponderance was particularly conspicuous in the age period from 20 to 29, when 81.8 per cent of all deaths among offspring of schizophrenic parents were due to tuberculosis, a number which was assumed to exceed greatly the mortality from tuberculosis in the population in the several regions in which the children lived. Figures are not given for the mortality from tuberculosis for this age period in the regions concerned. In the United States at present about 25 per cent of people dying between 20 and 29 die of tuberculosis. The mortality from tuberculosis in the children of the probants on which family records were based was estimated to be five times that of the general population in the second decade of life. Kallman did not make a detailed study of the probants themselves, but a gross estimate indicated that at least a fifth and probably a third of them had died of tuberculosis.

A hereditary factor appeared evident in the fact that the mortality from tuberculosis was highest in the descent group with the highest expectancy of schizophrenia. Various suggestions were made as to the biologic background for a union of such dissimilar characteristics as mental disease and susceptibility to tuberculosis, but none seemed concrete.

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54. Westphal, K., and Welti, M. H.: Klin. Wchnschr. **9**:1025, 1930.

55. Kallman, F.: The Genetics of Schizophrenia, New York, J. J. Augustin, 1938.

It is widely recognized that mortality from tuberculosis is high in institutions for mental disease because of the original presence of patients with open lesions, excessive crowding, bad hygienic habits and corresponding great opportunity for the spread of tuberculosis. Several analyses of the relation of mental disease and tuberculosis have been made in the light of this fact. Bogen, Tietz and Grace [56] concluded from a study in a large state hospital that differences in the tuberculosis morbidity and mortality rates for different types of mentally ill patients could be ascribed chiefly, if not entirely, to differences in the duration of stay in the institution and in the consequent exposure to infection. Their study did not support the theory that susceptibility to tuberculosis and predisposition to mental disease are constitutionally related.

Tuberculosis and Blood Groups.—A discussion of heredity calls for some consideration of blood groups. Numerous articles have been written on the association or lack of it between different blood groups and tuberculosis. As noted previously, Diehl and Verschuer [44] in their study of tuberculosis in twins found no special predilection of members of any blood type group to tuberculosis. Sasano's [57] conclusions on the basis of studies in New York were similar; no difference in initial susceptibility was seen, but slightly more favorable response to treatment occurred in members of group A. In England Bradbury [58] reported a concentration of members of group 4 (Jansky or Moss scale not indicated) among patients with tuberculosis as compared with the distribution of this blood group in the general population, but the figures and deductions are not convincing. In Russia Čikina [59] reported that the AB group had less than average resistance to tuberculosis; only 7.7 per cent of the population belonged to this group, but from 36 to 42 per cent of tuberculous patients in his experience were members of it.

In Argentina Celayo [60] reported that members of groups A and B were resistant and that a preponderance of tuberculous persons was found in group O. Few such patients were in the AB group, but these, like those in the O group, tended to have a doubtful or an unfavorable prognosis, with conspicuous caseation of lesions. In the Philippine Islands Bernardo and Medina [61] found the distribution of blood groups in tuberculous patients essentially the same as in the general population. However, members of the B group appeared the most prone to hemoptysis.

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56. Bogen, E.; Tietz, E. B., and Grace, M. F.: Am. Rev. Tuberc. **30**:351, 1934.

57. Sasano, K. T.: Am. Rev. Tuberc. **23**:207, 1931.

58. Bradbury, F. C. S.: Tubercle **16**:113, 1934.

59. Čikina, L.: Trudy permsk. med. Inst. **5**:140, 1935; abstracted, Zentralbl. f. d. ges. Tuberk.-forsch. **44**:410, 1936.

60. Celayo, M.: Rev. argent. de tuberc. **5**:49, 1939.

61. Bernardo, A. V., and Medina, F.: Bull. Quezon Inst. **1**:125, 1940.

It is evident from this brief selection of citations from different parts of the world that no striking or constant relation between blood groups and tuberculosis obtains. For other literature the reader is referred to Perla and Marmorston.[62]

RACE

Negro Tuberculosis.—The discussion of constitutional factors as related to race has concentrated largely on the question of Negro tuberculosis and has assumed an argumentative trend. The debate centers on the relative influence of constitution and environment. It is admitted by all investigators that in general the social and economic environment of Negroes favors the spread of tuberculosis, and in essence the argument is whether this alone accounts for the severity of the tuberculosis or whether a hereditary weaker capacity for resistance is concerned as well.

The argument on the basis of inherent racial difference is confused by the presence of two issues, one whether Negroes as a biologic type are less resistant than white persons, and the other whether they are less resistant because as a population they have had shorter contact with tuberculosis than has the white race and have therefore been less well selected with respect to this disease by the process of survival of the most fit.

Cummins,[63] who has made a lifelong study of tuberculosis in primitive peoples, has referred to the process of natural selection in the following graphic terms:

> I am inclined to picture an uninfected native community as being, and always having been, free from the tubercle bacillus; as having among its members the numerous non-resistant persons who would be cut off if tuberculosis were to be introduced, and as having, perhaps, a certain number who possess, though they do not use, the power to stop tuberculosis if it were present.
>
> All will alike live on and reproduce their kind as long as tuberculosis, that illness of civilization, is kept far away. But suppose that it arrives! All who meet it will equally get the infection and all, whether adults or children, will produce, if they sicken with it, the childhood type; there will be a much greater mortality than in a "habituated village" because the weaker in power to produce immunity, the weaker in power to resist the infection, will be very numerous in the community. And, with each fresh generation, there will still be the children of those who failed or who were not exposed, and it will take many generations to reach the immunity which exists already in the "habituated" community.

It may be assumed that in later years the number who originally possessed and, through lack of necessity, did not use the power to stop

62. Perla, D., and Marmorston, J.: Natural Resistance and Clinical Medicine, Boston, Little, Brown & Company, 1941.

63. Cummins, L.: Primitive Tuberculosis, London, John Bale Medical Publications, Ltd., 1939.

tuberculosis furnished the stock whence came the preponderance of survivors in the successive generations after tuberculosis was introduced. In his monograph Cummins has published a wealth of observation on the course and severity of tuberculosis in Negroes in Africa and placed the experience of others in significant relation to the whole picture of Negro tuberculosis.

In the early 1920's great interest was excited by the report of Borrel [64] on tuberculosis in the Senegalese troops brought to France in the World War. Few of these troops, coming from a region with little tuberculosis, had been exposed to tuberculosis previously. The overwhelming majority did not react to tuberculin. When exposed for the first time in France, they contracted serious tuberculosis on an epidemic scale. Not only was the disease more rapid and progressive than that to which French army surgeons were accustomed, but it was of an anatomically different type. Massive caseation of the lymph nodes and caseous pneumonia were characteristic and generalization the rule. A current description was, *"Il neige tuberculose"* (it snows tuberculosis).

Because of the resemblance of the Senegalese disease to the fulminant forms of childhood tuberculosis, the prevalent explanation was that primary tuberculosis had occurred relatively late in life in a people who had escaped the supposed protective influence of minor infection in childhood. The Senegalese troops were considered "virgin soil." For years afterward the experience was cited as evidence of the dangers of first infection in adult life.

The explanation of the severity and distinctive character of the Senegalese tuberculosis much more probably lies in the fact that the group possessed a high proportion of excessively susceptible persons, coming, as they did, from communities which had never been subjected to the process of natural selection by tuberculosis. In later years, as Cummins has clearly shown, it was discovered that native Africans reacted similarly even if they had had minor tuberculous infections in childhood. The only significant difference was that in the previously infected natives tuberculosis developed more rapidly under conditions favoring clinical progression, a fact presumably correlated with the hypersensitiveness due to previous infection. The type of tuberculosis seen among native Africans on the South African Rand, where Cummins' observations were made, was otherwise quite like that in the natives observed by Borrel, characterized by swiftly developing, generalizing disease, with "vast" enlargement and massive caseation of the tracheobronchial lymph nodes and miliary or caseonodular tuberculosis of the liver and spleen.

One other feature of tuberculosis in African natives particularly impressed Cummins, viz., their extraordinary sensitivity to tuberculin.

64. Borrel, A.: Ann. Inst. Pasteur **34**:105, 1920.

They reacted intensely to much higher dilutions than Europeans do. The excessive sensitivity appeared in no way an attribute of immunity, but rather the reverse. The more positive the reaction the greater seemed the likelihood of breakdown with tuberculosis, which Cummins believed was commonly endogenous, a result of strain in the work entailed, rather than exogenous. Cummins related the high degree of allergy to the biologic background, considering "sensitivity very great and immunity very low . . . characteristic of a stock still retaining a great number of persons unable to produce a good immunity to tuberculosis."

Epidemiologic Evidence.—In the United States and the West Indies the approach to the problem of racial variation in resistance to tuberculosis has been largely on an epidemiologic basis. Opie and his colleagues have carried out a long series of comparisons of the frequency and course of tuberculosis in Negroes and whites, which in essence indicate a predominant role of environment but leave open the explanation of certain differences in the anatomic characteristics of the disease in the two races. Commenting on the life tables of the two races for 1930, Opie [65] pointed out that in addition to the disparity in mortality rate—that for Negroes being three times that for whites—there was a difference in the mortality and morbidity curves for Negroes as compared with those for the white race; in Negroes tuberculosis of severe type began earlier in life and pursued a more rapid course. In Jamaica, with a population of 800,000 Negroes and 15,000 white persons, the differences between Negro and white tuberculosis as respects onset and course were of the same character but exaggerated. That is, the excess of the rate in Jamaica Negroes over the rate in white persons was greater than that observed in the United States, and the course of the disease was likely to be more rapid. The high rate in Jamaica was not to be attributed to recent introduction of tuberculosis in the Negro population, for there the Negro race had been in contact with the white race for more than three hundred years. On the other hand, the excessive severity could be attributed in large part without question to the crowding in dwellings and the careless habits of the people. In general, "massive dosage" of infecting bacilli was considered the main cause of the Negro's disadvantage.

In the succeeding ten years Opie and his associates added an abundance of confirmatory evidence to the early reports. Statistical studies by Putnam [66] showed that the annual incidence of secondary tuberculosis among Negroes exposed to tuberculosis was between two and three times that for white persons in contact. A larger proportion of Negroes than of white persons with household exposure acquired tuberculosis as

65. Opie, E. L.: Am. Rev. Tuberc. **22**:603, 1930.
66. Putnam, P.: Am. J. Hyg. **24**:536, 1936.

a result of this contact. In general, a much greater proportion of tuberculous Negroes had tubercle bacilli in their sputum, so that the intensity of exposure in Negro families was relatively great. Correspondingly, life after the onset of tuberculosis was comparatively short in the Negroes.[67] In a dispensary population of persons under observation for possible development of the disease, the ratio of the number with clinically manifest disease to the annual number who died of tuberculosis was 12.2 for white and only 4.3 for Negroes. One third of the white patients with clinically manifest tuberculosis had bacilli in their sputum, but sputum of this character was produced by one half of the Negroes.

In the most recent publications of this series [68] further stress has been laid on the rapidity of the course of Negro tuberculosis, the high rate of spread in Negro households, the relation of this rate to the frequency of lesions yielding sputum containing the bacilli and the speed of progression from latent infiltrative lesions, discoverable only on roentgen examination, to clinically manifest disease. Definite characteristic differences between the Negro and the white race were observed continuously in Philadelphia and in Jamaica with respect to different periods of life. These will be described in the section on age in this article.

The results of an investigation of a collaborating group in Alabama [69] were essentially confirmatory. Manifest tuberculosis was observed to begin earlier in Negroes, to progress more rapidly and to end in death more frequently than in white persons. The onset of secondary tuberculosis among Negroes was closely associated with the death of patients, a death often being followed by an explosive outbreak of the disease in the previously unaffected members of the household, a fact to be correlated with the great numbers of tubercle bacilli in the sputum in the last stage and the inadequacy of precautions against exposure in Negro homes.

On the basis of studies in North Carolina Donnelly [70] concluded that the evidence for lower resistance in Negroes than in white persons was more apparent than real. History showed that in the period of slavery, when modern competitive industrial conditions did not operate to their disadvantage, Negroes had as low a tuberculosis rate as white persons.

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67. Opie, E. L.; McPhedran, F. M., and Putnam, P.: Am. J. Hyg. **23**:530, 1936.

68. Saward, E. J.; Putnam, P., and Opie, E. L.: The Spread of Tuberculosis in Negro Families of Jamaica, B. W. I., in Saward, E. J., and others: Studies on Tuberculosis, The American Journal of Hygiene, Monographic Series no. 16, Baltimore, Johns Hopkins Press, 1941.

69. Graham, A. H.; Auston, P. W., and Putnam, P.: The Fate of Persons Exposed to Tuberculosis in White and Negro Families in a Rural Area of East Alabama, in Saward, E. J., and others: Studies on Tuberculosis, American Journal of Hygiene Monographic Series, no. 16, Baltimore, Johns Hopkins Press, 1941.

70. Donnelly, J.: Am. Rev. Tuberc. **31**:429, 1935.

Moreover, they share today in the continuing drop in the tuberculosis mortality rate, although the fall for Negroes is not as rapid as that for white persons. Donnelly called attention to the fact that primary tuberculosis commonly healed well in Negro children and that Negro adults often had forms of disease quite as chronic as those seen in the white race, and that this held for Negroes without white intermixture. He attributed the difference in end results in the two races in part to the lateness with which Negroes, with their relative insensibility to bodily discomfort, come under medical care.

Walsh and Mason [71] also ascribed the difference in fate of Negroes and white persons in part to psychologic differences, noting that tuberculosis as a rule does not cause pain and pain is a determinative factor in inducing Negroes to alter their manner of life and take advantage of medical attention. They emphasized the fact that disease commonly progresses to the far advanced stage in Negroes before they recognize that they are ill, and that even then they resist the restraint necessary for improvement.

Several studies with an epidemiologic approach have been made with the object of evaluating directly the influence of environment. Green [72] made an investigation of the relation of mortality to economic status in Cleveland, estimating the economic state on the basis of rent and the possession of luxuries. Two hundred and fifty-two small areas known as census tracts representing fourteen combined areas of varying economic level were studied. A powerful influence of environment on the mortality rate in both races became apparent. Green wrote, "The colored rate in each economic area was five times the white rate. The shape of the two curves was almost identical; that is, both white and colored tuberculosis death rates respond to economic conditions in a like manner."

Williams and Appelwhite [73] made a direct test of the influence of constitution and environment in a study of physical and economic factors in a group of Negroes in Georgia. The 843 subjects studied were divided into three physical groups, viz., those with strong negroid traits, those with strong white traits and those of intermediate character. About a third of the total number were tuberculous. The authors concluded that the incidence and the course of tuberculosis did not differ significantly in the different types of racial interbreeding. Certain physical differences found between tuberculous and nontuberculous patients were believed to be results rather than factors in casual relation to tuberculosis. On comparison of tuberculous and nontuberculous groups as to

71. Walsh, G., and Mason, H. M.: Am. Rev. Tuberc. **31**:413, 1935.

72. Green, H. W.: Tuberculosis and Economic Strata, Cleveland, Antituberculosis League, 1932.

73. Williams, G. D., and Appelwhite, J. D.: Am. J. Hyg. **29**:61, 1939.

immediate environment, the only difference found was in the higher proportion of known contacts in the tuberculous group. In conclusion, the development of tuberculosis was credited to the scattering of large numbers of tubercle bacilli, i. e., to the degree of exposure rather than to any other environmental factor or to individual constitution.

Clinical Evidence.—Numerous comparative studies have been made of the clinical course of tuberculosis in different races. Dickey [74] claimed that the race factor was not significant; in his experience children of the white, red, black and brown races reacted identically on first contact with tuberculosis. Because of the many uncertainties involved in comparing patients in different environments and different stages of tuberculosis, several authors have studied the course of tuberculosis in white persons and Negroes under supposedly equalized conditions. Bogen [75] reported that white persons, Negroes and Mexicans (i. e., an Indian-white mixture) in the same stage of the disease and under identical sanatorium conditions showed only insignificant differences in mortality rate, and hence he attached little weight to the factor of race in evaluating the expectation of survival. McCain [76] reached similar conclusions. On the other hand, Puffer, Stewart and Gass [77] found a consistently more rapid rate of progression and a consistently lower rate of improvement in all stages of tuberculosis in Negroes than in the corresponding stages in white persons.

A comparison of the development and course of tuberculosis under conditions believed nearly identical for the two races was made by Roth,[78] who studied a fifteen year peace time record in the United States Army. The group under examination consisted of males within a restricted age period, with the same equipment, standard housing, essentially identical rations and the same duties. The tuberculosis observed was believed to be, for the most part, that which escaped detection at the time of entrance examination. The disparity in mortality rates for Negroes and white persons under these conditions resembled the disparity for the corresponding groups in the general population; i. e., the rate for Negroes was four times that for the white persons. Admitted variables were the original environments of the soldiers of the two groups and the probable difference in their contact with open disease while in the army but on leave from military establishments.

Several recent studies have dealt with the clinical course of the disease in children and adults of the two races. Two will be cited.

74. Dickey, L. B.: Arch. Pediat. **52**:577, 1935.
75. Bogen, E.: Am. Rev. Tuberc. **24**:522, 1931.
76. McCain, P. P.: Am. Rev. Tuberc. **35**:25, 1937.
77. Puffer, R. R.; Stewart, H. C., and Gass, R. S.: Am. J. Hyg. **29**:894, 1939.
78. Roth, R. B.: Am. Rev. Tuberc. **38**:197, 1938.

Brailey [79] found the prognosis of tuberculosis in childhood to be intimately related to race. Negro children under dispensary care had three times the mortality of white children. Aside from age factors, to be discussed later, race itself appeared to be of great significance. The disparity in rate could not be attributed solely to excessive exposure in the Negro race, for children with known exposure did not show significantly higher mortality than those without known contact. Israel and Payne [80] compared the course of pulmonary tuberculosis in 265 white and 345 Negro adults consecutively found to have the disease in a tuberculosis clinic in a five year period. Qualitative differences in the character of the disease appeared significant. Approximately two thirds of the Negroes had predominately pneumonic and one third predominately fibrotic tuberculosis. For the white patients the figures were reversed. The onset was acute in Negroes, and death occurred in them more rapidly than in white patients with similar lesions. Basal tuberculosis occurred more commonly in Negro than in white patients. Negro patients reacted to tuberculin more strongly than white patients in every stage of the disease. In general, tuberculosis in Negroes was characterized by an acute course, but in those in whom the disease was fibrotic it often proved as chronic as in white persons.

Pathologic Evidence.—Reference has been made to the unique anatomic character of tuberculosis in African Negroes as described by Borrel [64] and Cummins.[63] Thorough studies have been reported from several countries. Describing the results of a series of necropsies in Philadelphia, Everett [81] wrote that pulmonary tuberculosis in Negroes as there observed differed from that seen in white persons in the following respects: Although originating in the apex in Negro adults as in white adults, the disease had a greater tendency to involve the tracheobronchial lymph nodes; it was more commonly pneumonic with massive excavation; it was less frequently found exhibiting a fibroid character, and less commonly observed as an incidental finding in the latent stage.

Pinner and Kasper [82] submitted a pathologic study which has been quoted widely as evidence for a true genotypic difference between Negroes and white persons in their response to tuberculous infection. Significant difference was apparent in three respects. First, in Negroes the lymph nodes were prone to be swollen to large size and to show virtually complete caseation, with occasional actual liquefaction, these features being as frequent in the reinfection as in the primary type. Second, the lesions in Negroes were exudative and less limited by normal anatomic boundaries; ulceration from the hilar glands into the

79. Brailey, M.: Am. Rev. Tuberc. **36**:347, 1937.
80. Israel, H. L., and Payne, H. M.: Am. Rev. Tuberc. **41**:188, 1940.
81. Everett, F. R.: Am. Rev. Tuberc. **27**:411, 1933.
82. Pinner, M., and Kasper, J. A.: Am. Rev. Tuberc. **26**:463, 1932.

bronchial tree and from an area of caseous pneumonia into the pleural space were much more frequent than in white patients. Third, generalized nodular tuberculosis occurred much more commonly in Negroes. The most conspicuous difference was in the Negro tendency toward involvement of the lymphatic system. Pinner and Kasper found it impossible to explain these differences on environmental grounds. They offered refutation of the environmentalists' argument that the excessive extent of tuberculosis in Negroes is simply the result of inoculation with a larger original infecting dose, citing evidence from animal experiments by Santo.[83] The latter studied the influence of dosage in corneal and subcutaneous infection of rabbits and guinea pigs and found that while great difference in the local intensity of the lesions resulted from variation in dosage, subsequent events, particularly the degree of involvement of the regional lymph nodes, were independent of the size of the infecting dose.

Certain studies from the West Indies indicate that the differences in anatomic character of tuberculosis between races may not be as great there as elsewhere. Koppisch [84] found no significant difference between white patients and patients with a mixture of white and Negro blood in Puerto Rico, a result agreeing with the clinical experience of Rodríguez Pastor and Ruiz Cestero.[85] Data on the anatomic character of tuberculosis in Negroes in Jamaica have been given by Wells,[86] without conclusions, however, as to differences from tuberculosis in white persons.

Tuberculosis in American Indians.—The literature on tuberculosis in Indians is scanty as compared with that on the disease in Negroes but significant in its record of high resistance. The current conception until recently has been that Indians were unusually susceptible to tuberculosis, but this conception was based chiefly on the high incidence of tuberculosis in Indian tribes, on which many published records agree (see Long [87] for a brief review). In a study of Indians in Minnesota Burns [88] found no marked differences in the clinical types of tuberculosis occurring among full blood Indians, mixed blood Indians and white persons. Long and Hetherington,[89] in a survey of tuberculosis in the Indians of southern Arizona, found that in the majority of those discovered to have advanced adult type tuberculosis the disease was of a

83. Santo, E.: Beitr. z. Klin. d. Tuberk. **77**:191, 1931.

84. Koppisch, E.: Puerto Rico J. Pub. Health & Trop. Med. **11**:492, 1936.

85. Rodríguez Pastor, J., and Ruiz Cestero, G.: Puerto Rico J. Pub. Health & Trop. Med. **11**:479, 1936.

86. Wells, C. W.: Am. Rev. Tuberc. **39**:796, 1939.

87. Long, E. R.: Nat. Tuberc. A. Tr. **31**:308, 1935.

88. Burns, H. A.: Am. Rev. Tuberc. **26**:498, 1932.

89. Long, E. R., and Hetherington, H. W.: Am. Rev. Tuberc. **33**:407, 1936.

chronic nature, conforming closely in roentgen appearance to the type of advanced disease usually seen in white patients. Korns,[90] in a study of Indians in New York state, found that resistance compared favorably with that of white persons in the same region, although the incidence of disease was much higher in the former. The fact that most of the Indians were of mixed blood was noted as a factor requiring caution in drawing conclusions on racial resistance. One differing character was the higher sensitivity of Indians to tuberculin. This had been recorded previously by Aronson [91] in a study of Indians in Michigan.

However, whatever the evidence on the nature of the response to tuberculous infection in Indians at the present time, it appears to have been a disease of far more acute course and of different anatomic character a half century ago. Ferguson [92] has published an impressive account of tuberculosis of intense epidemic character and high fatality that occurred in Indians of the great Canadian plains in the years following their concentration in reservation quarters in 1882. In the reservation studied two thirds of the deaths between 1882 and 1902 were attributed to tuberculosis. An enormous mortality occurred. At the peak of the epidemic the rate was 9,000 per hundred thousand or approximately three hundred times the present rate for the white population. A great decline in the incidence and severity occurred after the first two decades, and the evidence is good that natural selection removing the most susceptible stock was an important element in this decline. In the process of adaptation to civilization after 1882 one half of the family trees died out in three generations. In the families that passed out of existence 31 per cent of the deaths were due to tuberculosis, compared with 19 per cent in the families that survived. A study of nutrition, housing and sanitation, so far as this was possible by historical methods, suggested that these were of far less importance in the recession of tuberculosis than individual resistance to tuberculosis. Infusion of white blood regularly appeared to be associated with improvement in resistance. A preponderance of generalized, scrofulous forms of the disease, with caseation and ulceration of lymph nodes, was characteristic of the early phases of the epidemic.

Two questions arise in the evaluation of this interesting story, the validity of the records as respects tuberculosis and the relative influence of environment. Ferguson appeared satisfied as to the former. A great influence of environment must be admitted, for the entire way of life of the Indians was changed, with crowding, bad hygiene and great opportunity for massive infection. Ferguson compared conditions, however,

90. Korns, J. H.: Am. Rev. Tuberc. **34**:550, 1936.
91. Aronson, J. D.: Am. J. Hyg. **21**:543, 1935.
92. Ferguson, R. G.: Tr. Nat. A. Prev. Tuberc. **14**:5, 1928.

with some of those occurring in the worst period after the World War and concluded that while environmental changes were paramount as a cause of the postwar increase, they could not account entirely for the huge mortality of the Indian experience. "To those who are doubtful," he wrote, "of the increased susceptibility of primitive races to tuberculosis, I would say that no natural experiment among civilized people has approximated the intensity of these Indian epidemics."

It seems reasonable, in spite of the uncertainties, to interpret this Indian experience as an example of natural selection with survival of stocks resistant to tuberculosis. If this is correct, the selection was of rapid effect. Many such examples could be cited, however, from the experience of other Indians and other primitive peoples (Bushnell [93]). Anderson [94] estimated from a study in Mauritius that when a native race which has not been exposed to tuberculosis comes into continual contact with the disease the race begins to acquire resistance in thirty years, has it to an appreciable extent in fifty years and has it to a full degree in less than two hundred years.

Cummins [63] recorded an example:

> . . . later, with Dr. Welsh, to visit the Hospital. He has had 35 years' work at Umtata and has had numerous opportunities of studying tuberculosis in which he is much interested. He thinks that, while there is plenty of tuberculosis about, the type has changed during the last 10 or 15 years; and there are far fewer cases than formerly although more cases of illness are seen. Some years ago, he used to meet with instances of whole kraals being wiped out by spreading infection arising out of a single case of tuberculosis coming to live in the kraal. It is a long time since he has seen an instance of this: there seems more resistance now.

In a review of the voluminous early literature Perla and Marmorston [62] have cited numerous instances of the extermination of isolated groups of primitive people by tuberculosis.

Tuberculosis in Different White Stocks.—Traditionally, the Irish are of low and the Jews of high resistance to tuberculosis, with other groups in an intermediate position. Among the studies on this subject are those of Dublin and Baker [95] and Drolet.[96] From time to time investigations are reported confirming the sequence but not explaining the variation in the mortality rate. Putnam [97] studied the tuberculosis morbidity and mortality in 505 Italian and Jewish families attending a tuberculosis dispensary, with special reference to environmental factors. Factors of

93. Bushnell, G. E.: The Epidemiology of Tuberculosis, New York, William Wood & Company, 1920.
94. Anderson, D.: Am. Med. **35**:145, 1929.
95. Dublin, L. I., and Baker, G. W.: Quart. Pub. Am. Statist. A. **17**:13, 1920.
96. Drolet, G. J.: Bull. New York Tuberc. A. **4**:3, 1923.
97. Putnam, P.: Am. Rev. Tuberc. **28**:537, 1933.

this character that might have been significant were size of family and housing density. Subdivision of the families into those of 6 persons or less and 7 persons or more showed that racial differences in resistance were not detectable in large families. However, statistically significant differences were observed in small families. She wrote:

> One cannot escape the conviction that the type of tuberculous infection differed for the two races. Among the Italians the disease attacked a younger group and pursued a more severe, rapid and fatal course than it did in Hebrew families. The environmental factors considered in this analysis to which these differences could be attributed were the larger size of the Italian families and their greater housing density. The analysis on the basis of family size did not indicate that these were determining factors.

AGE

Age Selection.—In considering age it must be recognized that the progression of years by itself exerts a selective action and that if intrinsic differences in resistance to tuberculosis do occur within the human family, susceptibility may appear to be less in the late years of life simply because the less resistant stock has been removed by tuberculosis in the early years. There is reason to believe that individual variation in resistance does permit this kind of selection of more resistant stock for survival, but the mortality curve constructed on an age basis is complicated, and it is evident that the curve is affected by numerous factors. Certain age periods appear to have their special hazards, both environmental and constitutional.

Frost [98] presented arguments that the striking changes in mortality in the different age periods do not correspond with reasonably probable changes of like extent in exposure to infection and that inherent factors bearing on resistance must therefore apply. In his view there was little reason to believe that the remarkable rise in the tuberculosis morbidity and mortality rates in the years of adolescence and early adult life was associated with a corresponding increase in the rate of exposure. The apparent rise in the rate in the late years of life, on the other hand, must not be lightly assumed to be the result either of a decrease in resistance or an increase in exposure. The present mortality curve, as Frost pointed out, suggests that the greatest risk of death from tuberculosis for males occurs in the decade from 50 to 59 years, but if the mortality rate for the same cohort in their own earlier years is taken into account, it is seen that that cohort had a higher rate when its members were 20 to 29 years of age. In the meantime the tuberculosis death rate for all ages has dropped, and while men of 50 to 59 in 1930 had a higher tuberculosis death rate than men of 20 to 29 in that same year, they had a lower death rate than was experienced by the cohort of which they were members

98. Frost, W. H.: Am. J. Hyg. **30** (sect. A.):91, 1939.

thirty years previously, i. e., in 1900. On this basis it might be argued that the group as a whole had experienced an increase in resistance.

Rich,[99] among others, analyzed the life tables with respect to apparent resistance to tuberculosis at different ages and called attention to the necessity of relating the number of deaths not to the total number of persons living at any age but to the total number infected. He pointed out that, although infancy appears to be the most dangerous period of life in relation to the number infected, the factors of dosage and nutrition are so variable that no conclusions as to resistance can be drawn safely. In the period of 5 to 14, however, the mortality in proportion to the total number infected does seem to indicate age-specific resistance, for this is a decade with rapidly rising infection, as indicated by the tuberculin test, but with low initial and hardly increasing tuberculosis mortality. The sharp rise of tuberculosis in relation to puberty with no convincing evidence of a corresponding increase in exposure suggests genuine diminution in resistance at this period. There is a difference between the sexes in this respect, which will be considered in the section on sex.

Anatomic Differences in Tuberculosis at Different Ages.—As much attention has been devoted to the pathologic differences in tuberculosis in children and adults as to the clinical differences. It is currently accepted that as a rule pulmonary tuberculosis in childhood is associated with gross involvement of regional lymph nodes while that in adults is not and that the latter has a characteristic apical localization. It was formerly thought that the difference was due to the fact that in children the disease was primary and in adults it was the result of reinfection. The sensitization conferred by infection was supposed to have modified the response in the direction indicated in the disease resulting from reinfection. It was thought that if the time came when tuberculous infection was infrequent in childhood and common as a primary disease in adolescents and adults, the latter would exhibit the so-called childhood type, with midlung localization and enlargement of the lymph nodes. Actually experience has been highly variable in this respect (Israel and Long [100]). In all probability normal growth determines the character of response quite as much as previous infection with tubercle bacilli. Everett [81] advanced the view that apical localization of the infection is the result of physiologic changes occurring with the transition from childhood to adult life (see, however, the review, already cited, by Loeschke and Dehoff [20]), while involvement of the lymph nodes is a characteristic of the normal body lost after the modifying influence of infection has been exerted. The former is probably true, and there is growing evidence in the frequent absence of any involvement of lymph

99. Rich, A. R.: Minnesota Med. **21**:745, 1938.

100. Israel, H. L., and Long, E. R.: Am. Rev. Tuberc. **43**:42, 1941.

nodes in the primary tuberculosis of adults that a physiologic change inhibiting the development of tuberculosis has occurred in the lymphatic system as well as a change leading to localization in the apex of the lung.

An intermediate type of tuberculosis, with the ulcerative craniocaudal progression characteristic of the disease in adults and the caseation and enlargement of regional lymph nodes seen in children, was considered by Aschoff [101] to be characteristic of pulmonary tuberculosis in adolescents and designated *Pubertätsphthise*. Other pathologists and clinicians (Beitzke [102] and Redeker [103]) have not found reason to distinguish a special type of tuberculosis but agree that the disease often pursues an acute course in adolescents, with extensive caseous pneumonia.

Tuberculosis in Children.—That tuberculosis in the age period of 5 to 14 is clinically benign is universally agreed (for data see Tortone and associates [104]), but whether primary tuberculosis is benign in adolescent years is much debated (Long [105]; Israel and Long [100]). In this connection may be noted the statement by Sampson and Brown [106] that tuberculosis rarely develops in persons whose lungs are roentgenologically normal at the age of 25 years. The statement appears to have been an error, for contrary evidence from many clinics has since accumulated.

While tuberculosis occurring between the ages of 5 and 14 is usually benign, the common clinical experience is that it is somewhat less likely to be so in Negroes than in white persons. The difference in seriousness in Negroes and white patients, to which the preceding section on race was largely devoted, holds for all periods of life and is exaggerated for the periods of growth and early middle age in women. It holds likewise for infancy, as shown by Brailey.[79] Her experience brought out additional data on the risk of death in relation to the time of exposure. The first year of known infection was the most dangerous (see confirmatory view of Wallgren [11]); three quarters of the mortality in the white race and half of that in Negroes in the early years of life occurred within the year following first discovery of the infection. In both white and Negro infants the mortality was nearly twice as high for those known to be infected in the first 6 months of life as for those whose disease apparently originated between the ages of 6 months and 2 years. This is confirmatory of an old clinical impression, but Brailey's own work, in spite of careful consideration of the relation to exposure and environ-

101. Aschoff, L.: Verhandl. d. deutsch. Gesellsch. f. inn. Med. **33**:3, 1921; cited by Beitzke.[102]

102. Beitzke, H.: Ergebn. d. ges. Tuberk.-forsch. **3**:1, 1931.

103. Redeker, F.: Ergebn. d. ges. Tuberk.-forsch. **3**:41, 1931.

104. Tortone, J.; Chattas, A.; Myers, J. A.; Stewart, C. A., and Streukens, T.: Am. J. Dis. Child. **58**:92, 1939.

105. Long, E. R.: Arch. Path. **28**:719, 1939.

106. Sampson, H. L., and Brown, L.: Radiology **22**:1, 1934.

ment, left unanswered the question as to whether the fact was related to inherent susceptibility at the early age or to environmental factors.

Duration of Tuberculosis at Different Ages.—The studies of Opie and his associates on tuberculosis in white persons and Negroes have furnished important information on the relation between tuberculosis and age. They show clearly that with increasing age at the time of first observation of tuberculosis the length of time the disease lasts, before fatal issue, increases constantly. This holds not only for white persons but for Negroes despite the rapid advancement of lesions and the excessive mortality of the latter. The following tabulation adapted from Opie and Isaacs [107] illustrates this point:

Age at Time of Application for Dispensary Care (Yr.)	Duration of Disease (Mo.)	
	Philadelphia White Persons	Jamaica Negroes
15-19	25.5	8.5
20-24	28.3	9.3
25-29	26.9	9.5
30-34	40.5	12.8
35-39	42.8	13.6
40-49	53.3	21.0
Over 50	78.5	27.6

A relatively sharp increase in survival time is seen in both Negroes and white persons in the 30 to 34 year period.

Subsequent experience of the group of workers associated with Opie has confirmed the increase in survival time with later age of onset and brought out important relations between age and the rate of attack on contact. Putnam [66] found that the risk of acquiring tuberculosis within a specified period of observation was greatest for both white persons and Negroes when exposure began after the age of 15 years, was less for those first exposed during adolescence and was still less for those first exposed in childhood. Opie, McPhedran and Putnam [108] found that the danger of household contact is greater for adults than for children. Among white persons first exposed between birth and 14 years of age 1 in 20 acquired the disease, whereas among those exposed after the age of 15 years 1 in 15 became clinically tuberculous. In Negroes a corresponding observation was made, although the rates were higher; one twelfth of those exposed between birth and 9 years of age acquired tuberculosis, one ninth of those exposed between 10 and 14 years of age and one seventh of those exposed after 15 years of age. Subsequent observation confirmed this relationship, with accumulation of a large body of additional data. In general, the rates cited bear out the fact established by life tables and clinical observation that the period before puberty is one of relative resistance to tuberculosis and that after puberty one of relative susceptibility.

107. Opie, E. L., and Isaacs, E. J.: Am. J. Hyg. **12**:1, 1930.

108. Opie, E. L.; McPhedran, F. M., and Putnam, P.: Am. J. Hyg. **23**:530, 1936.

Tuberculosis in Aged Persons.—Until recently relatively little has been written with reference to tuberculosis in old age groups. The problem is complicated by the facts given at the opening of this section. As shown, there is reason to believe that the more susceptible of the exposed persons have been removed by death in the earlier age periods. It is probably true also that on the average old people are less likely to be exposed to tuberculosis than young people, although this is debatable. No sure conclusions can be drawn until environmentally similar groups are studied. If it can be accepted for present argument that both assumptions are correct, the rise in the tuberculosis death rate that is known to occur in the declining years of life may be taken as evidence of declining resistance.

Clinical observation, however, gives no substantial evidence for decreased resistance. Occasionally acute disease of bronchopneumonic or miliary character is encountered, but there is no statistical evidence that it is significantly more frequent than in persons of other age periods. According to Rubin,[109] miliary tuberculosis is rare as a clinical phenomenon in the aged but is a frequent unanticipated discovery at necropsy. In general, in Rubin's experience, as in that of Banyai[110] and that of Myers and Anderson,[111] tuberculosis in the aged is found to be of a fibrotic type, apparently of long standing, and, as might be expected, complicated more frequently than in young persons by nontuberculous disease.

Auerbach and Green[112] have recently reported an analysis of the results of 1,143 necropsies on tuberculous patients, about a third of which were on patients more than 40 years of age. In the group more than 40 years old there was a decrease in the number with acute disease, rather than a rise. Only 9.4 per cent of the 380 patients of the older group had acute or fresh tuberculosis. The intestines were less frequently involved secondarily than in the younger group, while the frequency of laryngeal involvement was about equal in the two groups. Hematogenous extension to the bones and urogenital system was not rare in the older group. Heart disease, diabetes and cancer were common in the older group.

Analysis of such reports suggests that death is not due to the tuberculosis alone and that when it is due to the tuberculosis it is the result of cumulative injury by the disease over a period of many years rather than of a drop in resistance to the tubercle bacillus as a specific result of increasing age.

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109. Rubin, E. H.: Am. Rev. Tuberc. **26**:516, 1932.
110. Banyai, A. L.: Am. Rev. Tuberc. **21**:568, 1930.
111. Myers, J. A., and Anderson, H. R.: Am. Rev. Tuberc. **21**:541, 1930.
112. Auerbach, O., and Green, H.: Quart. Bull., Sea View Hosp. **5**:237, 1940.

Experimental Studies.—Questions on the effect of age on resistance to tuberculosis might be expected to be answered satisfactorily by experiments in which environment could be controlled and tuberculosis of the same duration compared in old and young animals. A number of experiments of the desired character are on record. Neiman and Woolpert [113] obtained evidence by intracerebral inoculation of guinea pig fetuses that very young animals may be more susceptible than older animals to tuberculosis. In a large series of fetuses inoculated by this method with the almost avirulent B C G strain of tubercle bacilli more or less extensive generalized tuberculosis developed in the course of weeks after birth. Definite evidence was obtained that the B C G inoculum multiplied in fetal tissues.

Vorwald [114] also studied the course of tuberculous infection in fetal guinea pigs. Using the method of intraperitoneal inoculation, he obtained different results. The response in fetal tissues was definitely different from that observed in control adult animals. In a comparable time the fetuses usually showed less tuberculosis, even though relatively each fetus had received a larger dose in proportion to its weight. The cellular response in the fetuses was primarily mononuclear and epithelioid and not polymorphonuclear as in adult guinea pigs. Necrosis, which was prominent in the latter, was never conspicuous in the fetal tissues. Among animals that were allowed to live for longer periods or until they died from tuberculosis, the differences between the animals inoculated in utero and those inoculated in adult life were slight.

Smithburn [115] compared the course of tuberculosis after intracerebral inoculation of tubercle bacilli in young guinea pigs weighing 100 to 160 Gm., middle-aged ones weighing 380 to 480 Gm. and old animals weighing 860 to 1040 Gm. The survival time was short in each group, presumably because of the method of inoculation, but a definite difference was apparent in the three groups. The old animals survived for the shortest period, the middle-aged ones for an intermediate period and the youngest animals for the longest period. The number of animals was small. Such experiments should be repeated for confirmatory evidence. Laporte [116] found young chickens and guinea pigs more susceptible than adults to tubercle bacilli of low virulence.

A different approach was made by Freund,[117] who studied not only the amount of tuberculosis in animals of different age but also their response to tuberculin. Adult guinea pigs and guinea pigs 1 or 2 days

113. Neiman, I. S., and Woolpert, O. C.: Am. J. Path. **12**:153, 1936.

114. Vorwald, A. J.: Am. Rev. Tuberc. **35**:260, 1937.

115. Smithburn, K. C.: Proc. Soc. Exper. Biol. & Med. **38**:575, 1938; Am. Rev. Tuberc. **39**:383, 1939.

116. Laporte, R.: Compt. rend. Soc. de biol. **126**:667, 1937.

117. Freund, J.: J. Immunol. **17**:465, 1929.

old were inoculated with a large dose of tubercle bacilli. A month later all were tested with tuberculin both by cutaneous and by intraperitoneal inoculation. The amount required to kill varied in proportion to the weight of the animal, and the young and the adult guinea pigs showed a similar amount and character of tuberculosis. The chief difference was in the refractory state of the skin of the young animals, half of which did not react, while the others for the most part reacted only feebly. Valtis and Saenz [118] obtained similar results.

In other experiments Freund [119] learned that young animals are relatively deficient in the capacity to form antibodies, a fact that might have a bearing on resistance to tuberculosis in very early life. Experiments do not seem to have correlated resistance to tuberculosis with the high capacity of injured young animal tissues to undergo repair.

On the whole, the results of animal experimentation with respect to age and tuberculosis have not been highly informative.

SEX

Epidemiologic Evidence.—Comparison of the mortality curves of the two sexes shows that women have a higher tuberculosis death rate than men in the period of youth and early middle age and thereafter a lower rate. The relatively high rate in a restricted period has been the subject of continued inquiry. The approach has been chiefly statistical. Wolff [120] calculated that a close correlation exists between the tuberculosis rate and the fertility rate in the age period from 15 to 29. If in spite of the continued drop in the tuberculosis death rate at all ages young women between 15 and 29 continue consistently to have a higher tuberculosis mortality than men and women at higher ages, a close correlation with maternal activity is suggested. However, proof from the general population that deaths from tuberculosis are concentrated in young mothers is still lacking. Nicholson,[121] in a study of the homes of young women dying of tuberculosis in New York and Detroit, established a positive correlation between the onset of tuberculosis and recent pregnancy. The study was directed as much to changes in environment as to other causes for the high mortality, and her conclusions were that the excess of tuberculosis is essentially due to biologic rather than to environmental changes.

Menstruation and Tuberculosis.—A review of the whole question of sex and tuberculosis is given in a monograph by Jameson.[122] Attention

118. Valtis, J., and Saenz, A.: Compt. rend. Soc. de biol. **99**:1563, 1928.

119. Freund, J.: J. Immunol. **18**:315, 1930.

120. Wolff, G.: Am. J. Hyg. **30** (sect. A):63, 1939.

121. Nicholson, E.: Tuberculosis Among Young Women, New York, National Tuberculosis Association, 1938.

122. Jameson, E. M.: Gynecological and Obstetrical Tuberculosis, Philadelphia, Lea & Febiger, 1935.

is directed especially to the relation between menstruation and that between pregnancy and tuberculosis. The latter relation will be discussed later in this section.

An association has long been recognized between menstruation and fluctuation in the clinical course of tuberculosis. Sabourin wrote, "Tuberculous women are killed by their menses." Cough is apt to be more severe and expectoration increased. Body temperature increases, dyspnea may occur, and, if there is laryngitis, its symptoms may be exaggerated. These changes are believed to be associated with increased permeability of blood vessels around the lesions. According to Arnold,[123] the zone of tissue around a tuberculous focus is stimulated by the metabolic changes taking place normally between ovulation and menstruation, and the alterations that occur, including increased permeability of capillaries and cellular exchange, place the host with an active tuberculous lesion at a disadvantage.

Jameson [122] was not able to discover in roentgen films of tuberculous patients any changes that corresponded with the increase in symptoms at the time of menstruation. Amberson [124] noted that women with tuberculous bronchial stenosis suffered an increase in symptoms due to the stenosis regularly with each menstrual period. He attributed the exacerbation to turgescence around the lesion at that time.

A study by Johnston [125] on endogenous reinfection and the effect of sexual factors on tuberculosis appears to throw light on the manner in which the latter operate. A correlation was noted between puberty in girls, nitrogen balance in metabolism and breakdown with tuberculosis. Johnston studied normal and tuberculous girls before and after adolescence. The subjects were known to be out of contact with open tuberculosis. Puberty was found to have a depressing effect on the retention of nitrogen; higher amounts of protein than previously had been necessary were required to maintain the adolescent girl in nitrogen balance. Abnormalities in the calcium balance and basal metabolism were also evident. The diminution of reserves of nitrogen and calcium was believed significant for the onset of tuberculosis. Loss of calcium has long been considered a possible factor in tuberculosis (King-Turner [30]), but the relation to protein deficiency appears to be an approach deserving continued study. For older studies on adolescence and tuberculosis the reader is referred to Perla and Marmorston.[62]

The relation of menopause and tuberculosis has been much less investigated than that of tuberculosis and adolescence. Few writers seem to have been impressed by a connection. Bourgeois, Boquet-

123. Arnold, L.: Am. Rev. Tuberc. **28**:262, 1933.
124. Amberson, J. B.: Tr. A. Am. Physicians **55**:88, 1940.
125. Johnston, J. A.: Am. J. Dis. Child **59**:287, 1940.

Jesensky and Levernieux,[126] in studying 83 cases of tuberculosis, found 12 in which the development of fresh tuberculosis coincided with the menopause. In three quarters of these the subsequent course was slow and chronic. In 6 women preexisting tuberculosis appeared to be activated by the menopause. The changes observed occurred during the early stages of the period. Such reports are of interest, but unless the environment is thoroughly studied they cannot well be evaluated.

Experimental Studies on Castration and the Effect of Gonadotropic, Estrogenic and Androgenic Substances.—Numerous studies have been made of the effect of castration on the course of tuberculosis in male and female animals. These studies were carried out partly because of the sex difference apparent in the mortality curves and partly because of occasional reports of clinical benefit occurring in men and women with tuberculosis after surgical removal of the sex glands. It is a tradition that eunuchs are seldom tuberculous, although it seems unlikely that studies taking sufficient account of the degree of exposure have been made.

Early literature is reviewed by Bricker.[127] His own experience with a small number of animals led him to conclude that the course of tuberculosis is less severe in castrated rabbits of each sex than in noncastrated controls. Fabris [128] had similar results with female rabbits, also with a small number. Bourgeois and Boquet [129] found castrated male and female guinea pigs more resistant to tuberculosis than noncastrated controls. Other experiments are reviewed by Long and Vogt,[130] who studied the effect of castration of female mice on the course of tuberculosis induced a few weeks after the operation. It was their experience with several series of 50 mice each, infected with small doses of bovine type tubercle bacilli, that ovariectomized animals presented less severe tuberculosis and lived longer than nonovariectomized mice. With large doses of bacilli, no difference was seen between castrated and control animals.

Other investigators have found no significant effects of castration on the course of experimental tuberculosis. Bokkum [131] inoculated male and female guinea pigs five months after castrating them and compared the developing tuberculosis with that in control animals. No significant differences were noted in survival time or in anatomic character of the disease.

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126. Bourgeois, P.; Boquet-Jesensky, M., and Levernieux, J.: Rev. de la tuberc. **5**:546, 1939.

127. Bricker, F. M.: Ztschr. f. Tuberk. **40**:198, 1924.

128. Fabris, A.: Lotta contro la tuberc. **3**:1047, 1932.

129. Bourgeois, P., and Boquet, M.: Compt. rend. Soc. de biol. **128**:983, 1938.

130. Long, E. R., and Vogt, A. B.: Am. Rev. Tuberc., to be published.

131. van Bokkum, C.: Nederl. tijdschr. v. geneesk. **82**:3731, 1938; abstracted, Ztschr. f. d. ges. Tuberk.-forsch. **49**:569, 1939.

The reader is referred to Steinbach and Klein,[132] Gray and Brack [133] and Long and Vogt [130] for reviews of literature on the effect of gonadotropic, estrogenic and androgenic substances on the course of tuberculosis. In general the results are conflicting. Steinbach and Klein [132] reported that the gonadotropins of pregnancy urine and serum retarded the course of tuberculosis in rabbits and guinea pigs. Bourgeois, Boquet-Jesensky and Bourgeois [134] reported that an estrogenic substance accelerated tuberculosis in females. Gray and Brack [133] noted little effect from an estrogenic substance or from the gonadotropin in the urine of pregnant women, Long and Vogt [130] found in the experiments on mice to which reference has been made that ovariectomized mice treated with an estrogenic substance were less resistant to tuberculosis than untreated ovariectomized animals, the treated ones displaying about the same degree of resistance as normal females. No increase in the severity of tuberculosis was caused by giving excessive quantities of an estrogenic substance to infected normal females.

Further experiments seem necessary before conclusions can be drawn safely on the effect of estrogenic substances on the course of tuberculosis.

The effect of androgenic substances has been less studied. Thomas and Duran-Reynals [135] found that the addition of bull testicular extract to a suspension of tubercle bacilli increased the invasive capacity of the latter, so that the primary lesion and the general infection were more extensive than when tubercle bacilli were injected alone. Also the skin reaction to tuberculin was more intense if testicular extract had been mixed with the tuberculin before injection. Both of these effects were presumably due to the physical "spreading action" of the testicular extract rather than to a specific androgenic influence, although a report by Sylla [136] claimed that the injection of either an androgenic or an estrogenic substance enhanced the intensity of the tuberculin skin reaction in the homologous sex. Dermographism increased at the same time, and the effect was considered an influence on the vascular and nervous system intensifying general skin reactivity.

Bourgeois and Boquet [129] reported that testosterone increased resistance to tuberculosis. In their series of experimental animals the highest degree of resistance was found in noncastrated males given the androgen.

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132. Steinbach, M. M., and Klein, S. J.: J. Exper. Med. **65**:205, 1937.

133. Gray, C. B., and Brack, L. A.: Endocrinology **24**:645, 1939; **27**:322, 1940.

134. Bourgeois, P.; Boquet-Jesensky, M., and Bourgeois, D.: Rev. de la tuberc. **5**:754, 1939.

135. Thomas, R. M., and Duran-Reynals, F.: Proc. Soc. Exper. Biol. & Med. **31**:1201, 1934.

136. Sylla, A.: Klin. Wchnschr. **18**:311, 1939.

On the other hand, Carnes and Biskind [137] found no significant difference in the extent of tuberculosis in two sets of guinea pigs, one consisting of infected young normal controls and the other of similar animals with two pellets of an androgen planted in the skin of the back. The pellets exerted the expected physiologic effects, as shown by examination of the testes, but no visible action on the course of tuberculosis.

Thus in the case of androgenic substances the evidence for an effect on the course of tuberculosis is conflicting.

Pregnancy and Tuberculosis.—Few subjects in the field of resistance to tuberculosis have been more debated than the effect of pregnancy. A century and a half ago it was currently believed that pregnancy had a favorable influence on resistance. A swing to the opposite concept came with the special studies on tuberculosis by Louis and other French pathologists in the early nineteenth century. At present the trend is to think of the tuberculous breakdown which is frequently associated with pregnancy as not due so much to pregnancy as to the numerous concomitant and subsequent strains, including the extra burdens imposed before and after parturition, the economic changes, and the additional demands of lactation.

The various mechanical and physiologic ways in which pregnancy might have an adverse effect on the course of tuberculosis were reviewed by Jameson.[122] These include elevation of the diaphragm (now thought beneficial by some investigators), sudden decompression at the end of labor, increase in metabolic requirements, increase in lipoid content of the blood, maternal demineralization to supply the fetus, loss of fluid, endocrine disturbances and increase in capillary permeability.

A review by Lorenzetti [138] attributed unfavorable effects to increased calcium metabolism, vitamin deficiency, disturbance in endocrine equilibrium, anemia, toxic action of fetal metabolism, tendency to acidosis and accompanying respiratory disturbance. Lorenzetti stressed also the effect of changes in circulation involving the lung.

A review with extensive bibliography by Schultze-Rhonhof and Hansen [139] presents the current German point of view. These authors noted that the effect of pregnancy on tuberculosis is not considered as serious as it was once. They concluded that only certain types of tuberculosis are modified unfavorably.

As in other relationships with tuberculosis in which constitution might be concerned, environmental factors in the case of pregnancy

137. Carnes, W. H., and Biskind, G. R.: Bull. Johns Hopkins Hosp. **66**:297, 1940.

138. Lorenzetti, F.: Ginecologia **5**:75, 1939.

139. Schultze-Rhonof, F., and Hansen, K.: Ergebn. d. ges. Tuberk.-forsch. **3**:223, 1931.

introduce an almost insuperable obstacle to studies in man that satisfy the requirement of adequate control. In a number of studies in large sanatoriums, however, every effort has been made to take environment sufficiently into account. A few of the reports may be cited as evidence of recent and present trends in views in this country. Jennings and Mariette [140] concluded from a study of 470 cases that pregnancy does not have much, if any, effect on the progress of tuberculosis. The two conditions appeared without mutual effect. Skillen and Bogen [141] studied the records of 2,633 married women, of whom approximately half had borne one or more living children. The investigation was carried out on patients with varying pregnancy histories, with special reference to the extent of tuberculosis on admission to the sanatorium, the response to treatment and the effect of pregnancy on the future life expectancy as determined by the course of the disease after the patient had left the institution. The authors concluded that under proper care tuberculosis in women who become pregnant has a course not greatly different from that in patients not becoming pregnant and under similar treatment.

Ornstein and Epstein [142] studied the case histories of 82 pregnant tuberculous women; the majority had excavating caseopneumonic tuberculosis and a minority chronic proliferative tuberculosis. The investigation indicated that the prognosis depends on the character of the tuberculosis rather than on the advent of pregnancy. All of the patients with resolving exudative lesions and chronic proliferative disease responded favorably to treatment, while the course continued grave in patients with destructive lesions (Schultze-Rhonhof and Hansen [139]). Their final conclusion was that a group of nonpregnant females with similar lesions would have fared correspondingly.

The small amount of significant experimental evidence on record bears out the conclusions in this group of clinical reports. Burke,[143] for example, analyzed the records of 240 rabbits, 21 of which had given birth to one or more litters; of the 240 some had primary tuberculosis only and others primary and reinfection disease. The study failed to show any significant difference in the genesis of experimental pulmonary tuberculosis in rabbits which did and in those which did not give birth to young.

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140. Jennings, F. L., and Mariette, E. S.: Am. Rev. Tuberc. **25**:687, 1932.

141. Skillen, J., and Bogen, E.: J. A. M. A. **111**:1153, 1938.

142. Ornstein, G. G., and Epstein, I. G.: Quart. Bull., Sea View Hosp. **4**:420, 1939.

143. Burke, H. E.: Surg., Gynec. & Obst. **71**:615, 1940.

(To Be Concluded)

Notes and News

Appointments.—William Dock, professor of pathology at Stanford University Medical College and pathologist in chief to the Stanford University hospitals in San Francisco, has been named to the same positions, respectively, at Cornell University Medical College and the New York Hospital.

David P. Barr, professor of medicine at Washington University, has been appointed professor of medicine at Cornell University Medical College in the place of Eugene F. Dubois, who has been appointed professor of physiology and head of the department of physiology and biophysics at Cornell.

Society News.—The American College of Physicians will hold its twenty-sixth annual session in St. Paul, April 20-24, 1942.

Jesse L. Bollman, Rochester, Minn., is the new president of the American Society for Experimental Pathology; Baldwin H. E. W. Lucke is the vice president, and Harry P. Smith, the secretary.

Awards.—The Kober Medal of the Association of American Physicians has been presented to William de B. MacNider, Kenan research professor of pharmacology in the University of North Carolina.

At the Cleveland meeting of the American Medical Association, the Distinguished Service Medal of the Association was awarded to James Ewing.

Virus Laboratory.—The National Foundation for Infantile Paralysis has given $30,000 to the University of Michigan for the establishment of a laboratory for the study of virus diseases.

Enzyme Symposium.—A joint symposium on the respiratory enzymes and the biologic action of the vitamins has been arranged by the University of Wisconsin and the University of Chicago. The sessions on September 11 to 13, devoted largely to the respiratory enzymes, will be held at the University of Wisconsin, and the sessions on September 15 to 17 will be held at the University of Chicago. For information and reservations, write to T. R. Hogness, University of Chicago, Chicago, or Van R. Potter, University of Wisconsin, Madison.

Death.—Dr. Charles L. Connor, professor of pathology at the University of California Medical School, San Francisco, died June 12 at the age of 49.

Book Reviews

Hemorrhagic Diseases—Photo-Electric Study of Blood Coagulability. Kaare K. Nygaard, M.D., former fellow in surgery, Mayo Foundation; former assistant surgeon, University Clinic, Oslo, Norway; fellow of the Alexander Malthe Foundation for Research in Medicine, Surgery and Gynecology. St. Louis: C. V. Mosby Company, 1941.

This textbook represents observations on blood coagulation that have accumulated over a period of six years. The difficulties surrounding the interpretation of the physiology of coagulation are thus expressed in the preface: "With the constellation, however, of these three sets of problems with each of them harboring numerous imponderables, one is forced to juggle with three balls, two of which are constantly up in the air." The author points out that the maintenance of the fluidity of the blood contrasts strikingly with its coagulability and divides the factors involved in coagulability of the blood into intrinsic hematologic and external extrahematologic. "Coagulation is not a vital property of the blood but is a potential property."

The available methods for studying the coagulability of the blood are subjected to exhaustive analysis. The author cites the principles involved in each and the limitations. On rather presumptive evidence he concludes that the physical factors are essential for the coagulation of blood and arbitrarily discards Lampert's suggestion of the utilization of artificial resin products (bernstein and athrombit), which he regards as a refinement without purpose. In a word, he has been more interested in the constancy of external factors than in their refinement. In the progress of his studies he was struck by the inadequacy of human vision in matching colors and determining intensities of light. For this reason he directed his studies toward physical measures to standardize the method. He devised the photelgraph on the photoelectric principle, with a prospect of automatically measuring progressive changes in the coagulation of recalcified, citrated plasma. The plasma rather than the whole blood is used, for the obvious purpose of eliminating the formed elements. The graphic curves resultant in such studies are termed "coagelgrams." The curve shows a slight decline in the early seconds and then a precipitous fall. This is maintained for a very short period, and then there is a more or less gradual rise. The initiation of the precipitous curve marks the formation of the first fibrin needles as observed under the ultramicroscope. The lowest point in the curve indicates the transformation of the plasma to a gel. The rise in the curve indicates clot retraction. There is some lack of concurrence between the ultramicroscopic appearance and the contour of the coagelgram, which is explained by technical differences. On the basis of numerous studies, four periods of coagulation are outlined:

"1. Period of dissociation, comprising the interval from the beginning of the reaction to the first formation of fibrin.

2. Period of fibrin formation, comprising the entire, continuous process between points F and C of the coagelgram.

3. The rest period, between points C and R.

4. Period of clot retraction, beginning at point R."

The author stresses the importance of the velocity of the processes as an expression of the velocity of the formation of thrombin. Mathematical equations are used to indicate the availability of the photoelectric principle in quantitative estimates of blood fibrin. The author admits a lack of adequate support for the method (p. 93).

Part II of the text incorporates the influence of a series of variables, temperature, centrifugation, illumination and coagulant and anticoagulant factors, on

coagulation. The author points out that comparing the relative coagulability of citrated plasma, fixed standardized quantities of citrate per unit of volume must be used. He fixes the optimum level of ionized calcium at 1.6 millimols per kilogram of plasma. Citrated normal plasma has a coagulation time of 178 ± 3.6 seconds. The interaction of thrombin and fibrinogen and the coagulative effect of thromboplastin are carefully analyzed. It is pointed out that only occasionally is there actual fibrinopenia. In many instances of hemorrhage the fibrin is normal or increased. In general, according to the author, the rule holds that the velocity of the process of coagulation is governed by the first stage, namely, the formation of fibrin.

Part III of the text is devoted to a careful analysis of the application of these studies to the hemorrhagic diseases. The genesis and the mechanism of each are carefully and succinctly discussed. Table XX, on page 197, groups the hemorrhagic diseases primarily on the basis of their nonhematologic and hematologic origins and under the latter includes the number and functions of the platelets and the quantitative deficiency of fibrinogen, thromboplastin and prothrombin.

With regard to hemophilia, the author subscribes to the Sahli position of a deficiency of thromboplastin. Patients with this condition show a reduced velocity in the first as well as the second stage of the process. The author holds that there is no adequate evidence for the rejection of the theory of a qualitative deficiency of platelets. In the discussion of thrombopenic purpura he adduces evidence for a pronounced hypocoagulability of the blood. Two chapters are devoted to (1) the hemorrhagic tendencies in diseases of the gallbladder, bile ducts and liver and (2) the hemorrhagic diseases of the newborn. In these two chapters the recent literature has been carefully analyzed and reconciled with the work of the author.

Errors are very infrequent in this excellent text. However, the date in the heading "woman (born in 1922)" (p. 229) must be incorrect. On the same page, the name Leede is spelled Lede rather than correctly as found in the references at the conclusion of the chapter.

The author is particularly commended for his observance of scientific amenities, as witness the following quotations from pages 44 and 116, respectively:

"At the time of Kugelmass' interesting investigations, the reliability of the photo-electric measuring devices was not such as to form the basis of more accurate investigations. His experimental setup could hardly have prevented him from working out the subsequent photoelectric investigations if satisfactory instruments had been available at that time."

"To avoid misunderstanding, let it again be emphasized that my intention has not been to attempt a substantiation of the investigations of the other workers. I have desired only to use their results as a yardstick for the applicability of my technique to the present problem."

This magnificent monograph will recommend itself not only to students in the field of hematology but also to physiologists and clinicians generally interested in the subject of blood coagulation. The method on which the author has capitalized will find a much wider application than the limited field to which he has applied it. He is to be congratulated on his fine contribution.

A Textbook of Clinical Pathology. Edited by Roy R. Kracke and Francis P. Parker, Emory University, Atlanta, Ga. Ed. 2. Pp. 780. Price $6. Baltimore: Williams & Wilkins Company, 1940.

The appearance of a second edition after two years indicates that this book is filling a need. The increase in the number of pages from 567 to 780 shows that the editors and authors are striving to improve the book and to keep it abreast with developments. The general makeup of the book is retained, but the first two chapters of the first edition, on laboratory equipment and on solution and other related topics, have been placed at the end as four appendixes.

New subjects in this edition include, among others, arterial puncture, sternal puncture and biopsy, the differential test for infectious mononucleosis, newer data

on prothrombin, leptospirosis and spiroplasmosis, trichinosis, determination of phosphatase and of the new chemotherapeutic substances of the sulfonamide group, examination of duodenal contents and of seminal fluid, preparation of autogenous vaccines and the quantitative Friedman test. Two valuable new chapters are added, one on the diagnosis of venereal lesions and the other on assays of hormones and vitamins. The latter is a clear presentation of a difficult subject in the brief space of 22 pages. The chapter on serologic tests for syphilis has been completely rewritten and brought up to date.

All these changes, additions and innovations have contributed to make this actually a new book and not merely a new edition. Fourteen authors collaborated in reviewing: modern clinical pathology, including the quantitative, the morphologic, the chemical and the serologic examination of the blood and of other fluids; clinical bacteriology and parasitology; excreta and secreta; transudates and exudates; function tests of various organs. Especial emphasis is placed on the interpretation of the results and on the theoretic background of the tests, but technic has not been neglected. There are no unnecessary repetitions, and the frequent cross references to different chapters evidence careful editing.

A few minor errors may be pointed out, all easily correctable in the next edition. It is stated on page 218 that "the presence of agglutinins in saliva and in seminal fluid, identical with those in the blood serum, makes examination of stains due to these substances valuable as a method of identification in criminal cases." It is the presence of agglutinogens which is generally considered the more important factor. A statement on page 213, dealing with cross agglutination, may be misleading: "If no agglutination occurs on either side, the two bloods are said to be compatible and therefore may be used for transfusion. If, however, agglutination in any degree is present on either side, they are designated as incompatible and must not be used." When a donor of group O is used for a recipient of another group there will be, as a rule, agglutination of the cells of the recipient by the serum of the donor, without necessarily excluding such a donor. The Weltmann and the Takata-Ara tests are not considered.

The illustrations, 34 full page plates, 23 in colors, are well chosen. The presentation is on a high level, with such variations in style as are unavoidable with different authors. Bibliographic lists at the ends of chapters have been brought up to date. The binding, the paper and the print are of high quality. The index is well arranged and shows evidences of careful revision; it has grown from 13 pages in the first to 30 in the present edition.

Medical students and physicians will find this a valuable book. The indications for all important laboratory tests, the interpretations of laboratory results, and, what should be of special value, the discussion of limitations of laboratory procedures are clearly presented. The book will be useful to the clinical pathologist for quick and ready reference; the first ten chapters (219 pages) contain an excellent discussion of hematology. The medical technologist will find the book a trustworthy guide in modern medical technology.

Bacteriology in Neuropsychiatry. Nicholas Kopeloff, Ph.D., research bacteriologist, New York State Psychiatric Institute and Hospital, New York. Cloth. Pp. 316. Price $4.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1940.

This book by a research bacteriologist is a survey of investigations concerned with the specific role of infections and immune processes in nervous and mental diseases. The parts dealing with etiology, epidemiology, animal experiments and immunology are excellent, but some portions pertaining to pathology and more particularly to symptomatology are not up to the high standard set by the purely bacteriologic portions of the text.

Speaking of tuberculosis, for example, the author states, "The presence of tubercles in the brain gives rise to severe neurologic disturbances, such as ataxia, pareses, convulsions, hemiplegia and myelitis." Speaking of the neuropsychiatric

manifestations of typhoid and paratyphoid fever, he lists "dizziness, headache, clouding of the sensorium, stupor, maniacal attacks, meningismus, hemorrhagic pachymeningitis, brain abscess, cerebellar ataxia, and hemiplegia as well as peripheral and cranial nerve involvement, disturbances in the vegetative nervous system and spondylitis." Such statements can be of value to no one.

The third portion of the text dealing with "diseases of unknown etiology involving the central nervous system" contains discussions of the role of autointoxication in mental disease and the possible role of toxins and tuberculosis in schizophrenia. Other chapters deal with those two diseases with an enormous literature—epilepsy and multiple sclerosis.

This survey gives evidence that bacteria and their products play a minor role in the etiology of mental diseases, especially in the major psychoses.

The book closes with an excellent study of anaphylaxis, serum sickness and allergy as they pertain to the nervous system. The author index contains over one thousand names.

A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students. Edwin E. Osgood, M.A., M.D., associate professor of medicine and head of the division of experimental medicine, University of Oregon Medical School, and member of the staff of Multnomah County Hospital and of the consulting staff of the Doernbecher Memorial Hospital for Children, Portland, Ore. Third edition. Cloth. Pages 676, with 27 figures in the text and 10 colored plates. Price, $6. Philadelphia: The Blakiston Company, 1940.

The appearance of a third edition in the course of nine years indicates that this textbook continues to fill a need. The subject matter of the previous editions was reviewed in detail in former issues of the ARCHIVES OF PATHOLOGY (**12**:863, 1931; **22**:133, 1936). The present edition is enlarged by 91 pages, which are distributed as follows: fifty-seven pages have been added to the first part, which is theoretic, 12 pages to the second part, on methods, and 11 pages to the index. An author index has been supplied. Some chapters have been rewritten and brought up to date, and descriptions of several new procedures have been added.

Most of the laudatory remarks of the reviewers of the previous editions can be applied to the present one. However, an innovation has been introduced which detracts seriously from the value of the present edition. The author's nomenclature of the cells of the blood and bone marrow has brought in a highly controversial subject. There is no doubt about the existing confusion in hematologic terminology, but one questions whether a new nomenclature with many artificial terms will do anything but add to the confusion. The history of science and of medicine in particular teaches that uniformity of nomenclature has never been established by a decree. It is feared that students may be perplexed and that the advanced reader will find it difficult to accept the new system. In either case the innovation does not seem to be a lucky one.

The revision has failed to eliminate some of the minor errors of the second edition, for instance, a period in the middle of a sentence on page 8, a wrong spelling of "aplastic" on page 207, a page reference to the text (to page 519 instead of to page 527) in the schedule of assignments on page 578, and a reference to the Friedman test in the subject index.

Books Received

INFORME DE LA JUNTA DE BENEFICENCIA DEL DISTRITO FEDERAL Y DEL INSPECTOR GENERAL DE LOS HOSPITALES CIVILES DEL DISTRITO FEDERAL. CORRESPONDIENTE A SUS ACTIVIDADES EN EL LAPSO COMPRENDIDO DEL 1° DE DICIEMBRE DE 1939 AL 30 DE NOVIEMBRE DE 1940. Pp. 192, with charts. Caracas, Venezuela: Tipografia la Nacion, 1941.

MALIGNANT DISEASE AND ITS TREATMENT BY RADIUM. Stanford Cade, F.R.C.S.; Surgeon, Westminster Hospital, Mount Vernon Hospital and the Radium Institute; lecturer in surgery, Westminster Hospital Medical School; associate examiner in surgery, University of London; late Hunterian professor and Arris and Gale lecturer, Royal College of Surgeons of England; member of the Grand Council of the British Empire Cancer Campaign. Pp. 1291, with 623 illustrations, many in color. Price $18. Baltimore: Williams & Wilkins Company, 1940.

NATURAL RESISTANCE AND CLINICAL MEDICINE. David Perla, M.D., late pathologist and bacteriologist to Montefiore Hospital, New York, and instructor in medicine, Columbia University College of Physicians and Surgeons. Jessie Marmorston, B.S., M.D., formerly bacteriologist to Montefiore Hospital, assistant in pathology, Cornell University Medical College. Pp. 1344. Price $10. Boston: Brown & Company, 1941.

HISTOLOGICAL STUDIES ON THE NORMAL AND THE IRRADIATED SUPRARENAL GLAND IN RABBITS: A CONTRIBUTION TO THE SUBJECT OF SEASONAL CHANGES IN THE ADRENAL CORTEX AND OF THE DIFFERENTIATION OF THE CORTEX CELLS. Olav Torgersen. Pp. 112, with 12 tables and 7 plates. Oslo, Norway: Jacob Dybwad, 1940.

PHYSICAL MEDICINE. Frank H. Krusen, M.D., F.A.C.P., associate professor of physical medicine, Mayo Foundation, University of Minnesota; head of the section on physical therapy, Mayo Clinic; member of the Council on Physical Therapy of the American Medical Association; past president of the American Congress of Physical Therapy; past president of the Academy of Physical Medicine. Pp. 846, with 351 illustrations. Price $10. Philadelphia: W. B. Saunders Company, 1941.

MEDICAL CARE IN NEW YORK STATE, 1939. REPORT OF THE TEMPORARY LEGISLATIVE COMMISSION TO FORMULATE A LONG RANGE STATE HEALTH PROGRAM. Pp. 492. Albany: J. B. Lyon Company, 1940.